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Accession No. 3202

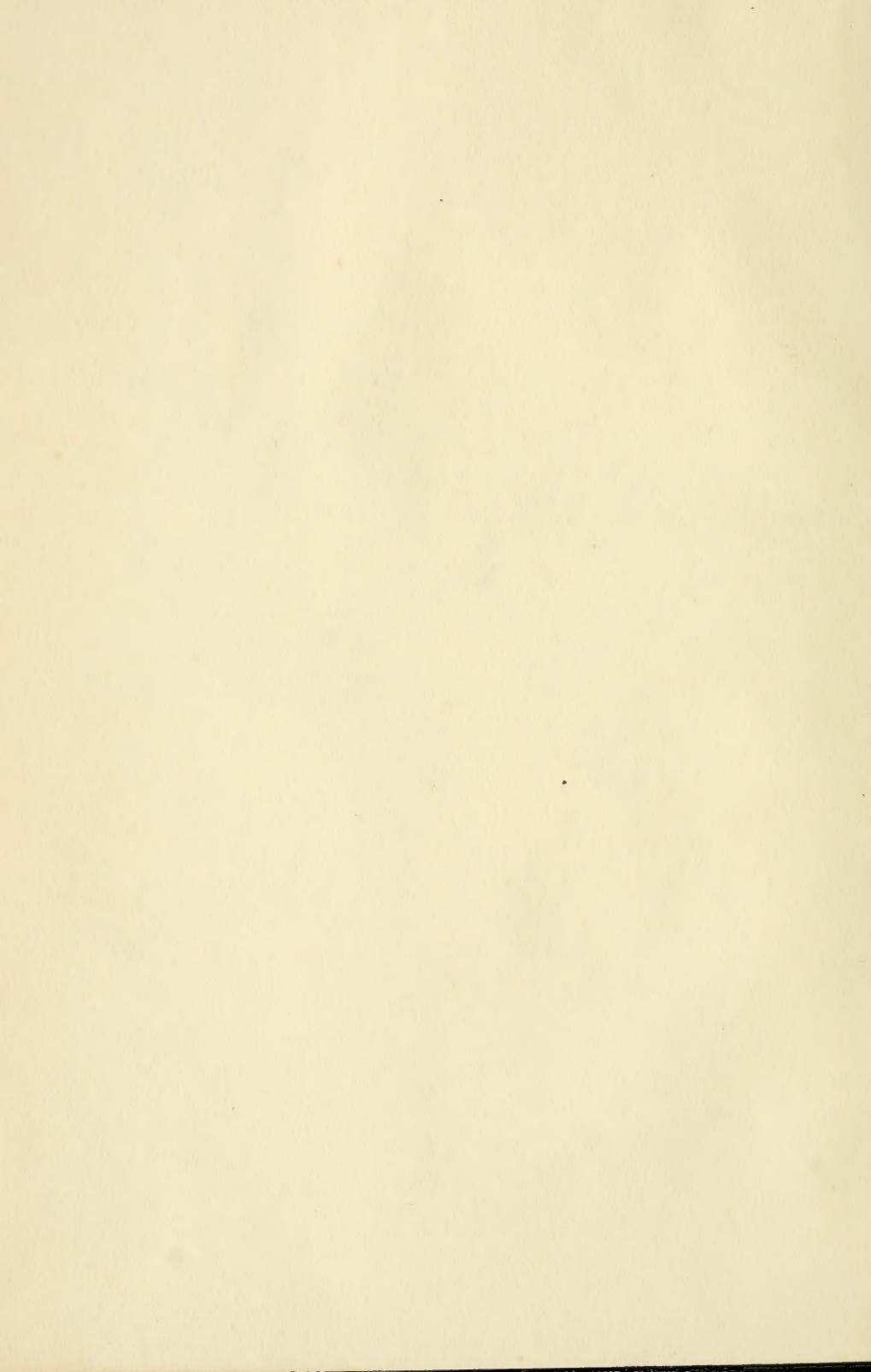
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VOLUME IV

1905

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THE AMERICAN JOURNAL OF ANATOMY
BALTIMORE, MD., U. S. A.



The Friedenwald Company
BALTIMORE, MD., U. S. A.

1220

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NOTE.

Table 15, p. 322, summarizes 60 cases. The figures under the headings "Common trunk" and "Absent" do not enter into the totals in the last column, which were made by adding the figures in the other columns. In the fifth line the number of cases (3) in which the artery occurred double should be added, making the total 62 instead of 59.

Table 16, p. 323, summarizes 69 cases. The figures under the headings "Common trunk," "Absent" and "A. cervicalis superficialis" do not enter into the totals given in the last column. In the line opposite "A. cervicalis ascendens" transpose 9 and 5.

The total number of cases in which any given artery has been worked out is often less than the total number of cases dissected, because for various reasons it was impossible to work out each individual artery in every case.

ON THE DEVELOPMENT OF THE BLOOD-VESSELS OF THE BRAIN IN THE HUMAN EMBRYO.

BY

FRANKLIN P. MALL.

From the Anatomical Laboratory of the Johns Hopkins University.

WITH 3 DOUBLE PLATES AND 4 TEXT FIGURES.

During the past year, while studying sections through the heads of the embryos in the collection at this laboratory, it was noticed that in some of the specimens the blood-vessels were unusually well marked, for they were well distended with blood. This natural injection made it possible to reconstruct the blood-vessels in a satisfactory manner down to the capillaries. At the same time I obtained from Mr. Brödel a number of embryos' brains in which the arteries had been injected with Prussian blue, which, together with numerous embryo pigs injected alive or immediately after death, form the basis of this study.

TABLE OF EMBRYOS STUDIED.

Number.	Length in mm.	Thickness of Section in μ .	Direction of Section.	From Whom Obtained.
2	7	15	Transverse	Dr. C. O. Miller.
163	9	20	"	Dr. D. S. Lamb.
109	11	20	"	Dr. Harvey Cushing.
144	14	40	Sagittal	Dr. Watson.
74	19	50	"	Dr. Irving Miller.
145	33	50	"	Dr. W. T. Watson.
225	46	..	Injected	Dr. Wegetarth.
237	48	..	"	Dr. Todd. (Brödel Collection).
235	59	..	"	Dr. Linthicum.
234b	65	..	"	Brödel Collection.
..	80	..	"	Brödel Collection.
234a	80	50	Transverse	Dr. Ashby (Brödel Collection)
233	90	..	Injected	Dr. Smart "
236	92	..	"	Dr. Wilson "

The blood-vessels of five human embryos were reconstructed from serial sections, and eight older embryos which had been injected were dissected. The brains of pigs which had been injected with India ink proved to be of great value to control the studies of the human. It is quite easy to make single or even double injections of young embryos by injecting them either before or after death, or both. In case India ink is injected into the liver of a live pig with a hypodermic syringe, the

fluid is taken up by the heart and is pumped through the arterial system. When all the arteries are full the beat of the heart may be arrested by cooling the embryo. A second injection into the liver with a different fluid (and for this purpose I usually employed aqueous Prussian blue) fills the entire venous system. More frequently single injections were made of the arteries or of the veins by injecting India ink into the liver either before or after the heart had ceased to beat. India ink, being resistant, is preferred, for embryos injected with it can be hardened in alcohol and cleared in a one per cent solution of caustic potash and preserved in glycerine. Such specimens are perfectly transparent, showing the arrangement of the vessels beautifully and their relation to the structures within the head. Sagittal sections of whole embryos are also very valuable for study, for the half brain is easily peeled out, leaving the injected membranes intact within the head.

It is difficult to make complete injections of the veins of the head in dead embryos without extravasations into the arachnoid spaces. So frequent is this extravasation that one is inclined to think that the vessels of the brain, especially the veins, have open communication with these spaces. But since the arachnoid spaces are always free from blood, and since complete injections with India ink made by the contraction of the heart in live embryos do not form extravasations, it must be concluded that the vessels are closed in life. A similar communication has been demonstrated by Key and Retzius¹ in the adult brain by injecting Prussian blue into the arachnoid spaces. Frequently the fluid passes over into the sinuses through the Pacchionian bodies, showing that here again the communication is easily established. This question will be taken up again in the description of the specimens.

I shall first describe the blood-vessels of the brains of eight embryos of the third month, which had been injected, then take them up in regular order, beginning with a reconstructed specimen of the fourth week. At this point I wish to express my great obligations to Mr. Brödel for much of this valuable material. He has an exceptional opportunity to obtain many fresh specimens which can be injected, and I sincerely hope that physicians will continue to send him all the embryos they obtain, for Mr. Brödel makes the greatest possible use of them.

INJECTIONS.

Unfortunatley there is a tendency for the Prussian blue which has been injected to extravasate over the surface of the brain, interfering

¹ Key and Retzius: *Studien in der Anatomie des Nervensystems und des Bindegewebes*. Stockholm, 1875, p. 218.

very much with the sharpness of the arterioles and making it impossible to define the veins, or embryonic sinuses. So constant is this extravasation in position and degree that it often seems as if the arachnoid spaces communicate freely with the veins, but, as will be shown presently, this is not the case.

In the smallest specimen (No. 225, 46 mm. long) the middle cerebral artery and the arteries to the mid-brain are well injected, but in no case does the injection extend into the brain substance. The arachnoid spaces are filled evenly with the blue injecting fluid, but there is none within the ventricles. Since the fluid does not reach the capillaries, it is evident that the extravasation took place from the arterioles, and this seems to be the case, for the arterioles are easily torn at the point they enter the brain substance. In the early stages the brain is attached only slightly to the embryonic pia mater, and it is practically impossible to remove the brain with its pia mater intact, as can be done in older embryos or in the adult. At the point the vessels leave the pia mater to enter the brain substance the blood-vessels have but a single endothelial wall, and it is here that the rupture and extravasation take place when these arteries are injected.

In an embryo a little older, No. 237, Fig. 1, the injection of the artery is practically perfect, and I have therefore given a drawing of it. The brain was peeled out with its pia mater only with difficulty and over the region of the lateral cerebral fissure (Sylvius) some of the vessels separated and remained attached to the dura. This portion was drawn inverted and redrawn upon the brain, and the point at which the main trunks are torn off is indicated in the drawing in the region of the island. The injection is practically a complete arterial injection with but little extravasation into the arachnoid and none into the ventricles. An extravasation is over the region of the island, on both sides, and to a slight extent over the mid-brain on one side. The arteries divide and subdivide in regular fashion until the terminal branches are reached, when they turn at right angles to enter the brain substance. There are from five to ten of these cortical arteries to each square millimeter of brain surface. Around some of them there is some extravasation of Prussian blue, indicating the way the blue enters the arachnoid spaces.

Over the surface of the brain of an embryo 65 mm. long (No. 234^b) there are numerous blue spots, about one to each square millimeter. Where the spots are larger there is a tendency for them to run together, but in general the brain is only spotted rather than being covered evenly with an extravasation. There is no extravasation in the ventricle. In another brain of about the same age (No. 235, 59 mm. long) the ex-

travasation is complete, filling all the arachnoid spaces and the whole ventricle. After the extravasation was brushed off, the brain substance was still found to be spotted, showing that the extravasation penetrated the brain substance.

In an embryo of the beginning of the fourth month (80 mm. long) the whole brain was evenly spotted, about one spot to each square millimeter. Another specimen of the same age and of the same general appearance (No. 234^a, 80 mm. long) (Fig. 2) was cut into serial sections in order to study the relation of the spots to the surrounding tissues and to the cortical arteries. Around the large cortical arteries (possibly the medullary arteries) there is an extravasation which encircles the vessel as a small spherical body. There is no rupture of the vessel. It indicates that at this point the vessel is at least very pervious. There is no extravasation into the ventricle.

In specimen No. 238 (90 mm. long) both the arteries and the veins were injected without injecting the capillaries. There was no injection of the brain substance, and there is no extravasation of the cortex nor into the ventricles. At the base of the brain and in the falk there is considerable extravasation, apparently coming from the veins. In an embryo of the same age (No. 236, 92 mm. long) the arterial injection is complete again, with the usual spots of extravasation in the cortex of the cerebral vesicle. The extravasation fills all of the arachnoid spaces as well as the cavities of the ventricle. The injection passes through the medial opening into the fourth ventricle (Majendie), and apparently the ventricles are injected through this opening from the arachnoid.

It is apparent from the description of the injected embryos that as a rule the extravasation into the arachnoid spaces takes place from the arteries as they penetrate the cortex of the brain, and that in case the veins are injected the extravasation is directly from them. This conclusion was reached in part by making corresponding injections of embryo pigs, many being constantly at my disposal. In general the extravasation is the same in the pig as it is in the human embryos. It frequently appeared, however, as if the India ink injected leaked with even greater ease from the veins and sinuses of the pig's brain. In embryos in which the heart had just stopped beating the injected fluid would first fill the jugular veins, then the sinuses, from which the arachnoid spaces filled as readily as did the capillaries.

When the arachnoid spaces were filled by injecting directly into the lateral ventricles of perfectly fresh embryos, the injected fluid would not pass over into the veins. I made this test repeatedly with live embryos from 3 to 8 cm. long, always with the same result. It is best to inject

ordinary India ink into the ventricle of a live embryo with a hypodermic syringe. The ink spreads at once throughout the central canal of the brain and cord and escapes through the medial opening of the fourth ventricle and fills the spaces of the arachnoid of the whole brain and cord. From the cord the ink extended for a short distance along the main trunks of the spinal nerves. In the larger embryos the ink invariably flowed freely from the mouth of the pig as soon as all of the arachnoid spaces had been filled. After hardening the specimens in formalin, razor sections showed that it had reached the mouth through the Eustachian tube. It had entered the middle ear along the trunks of the seventh and eighth nerves. In younger embryos (5 cm. long) the fluid came out of the mouth in only half of the tests, while in the smallest ones injected (3 cm. long) it did not come out of the mouth at all.

In all of these tests the India ink or the Prussian blue should have passed over into the veins were the communications with them free. In all instances the pigs were still alive or just dead when the tests were made, for it is known that extravasations take place with the greatest of ease after the embryo has been dead for some time. While in these tests injections could be made with ease from the veins into the arachnoid spaces, but not in the opposite direction, in embryos still alive it was found that in no instance would an injection into the artery pass into the arachnoid spaces. The live embryo may be injected using its own heart to inject the India ink. If the uterus is kept warm the embryo will remain alive for an hour or longer, giving ample time. The ink is to be injected directly into the liver with a hypodermic syringe and then by means of gentle massage or by gravity it is forced into the heart, which gradually pumps it all over the body. The arteries to the brain fill slowly and the granules pass over into the veins. If at this time the embryo is cooled the heart will stop, thus giving a single injection of the arteries. If it is continued, the veins will fill through the capillaries, which confuses more or less. Yet this double injection is desired in this test, for the result is always the same: in no instance is there an extravasation into the arachnoid space. In case too great a quantity of ink is injected into the liver, it is forced directly into all of the veins of the body and then the ink granules will leave the veins and enter the arachnoid spaces. If the injection of the arteries and veins of the brain is made through the arteries, using the embryo's heart to do the pumping, all of the granules remain within the blood-vessels, showing conclusively that there are no free communications between the vessels and the arachnoid spaces. When the granules do leave the spaces by injecting them directly into the veins, we must conclude that artificial openings are

made in their walls by the excessive pressure, no matter how careful we are in making the injection.

The delicate veins and capillaries can be injected without extravasation of the fluid in case it is done by injecting a small quantity of India ink into the liver and allowing it to run from there to the head by gravity. In this way I have often obtained beautiful specimens which are clear and sharp. This pressure, which is often not over one centimeter of water, is so small that it cannot possibly be imitated with a syringe. In fact it is similar to that produced by the embryo's heart, and with these normal pressures no extravasation takes place.

I may add that in all cases the embryos were placed at once in the strongest alcohol in order to prepare them so that they may subsequently be cleared in a one per cent solution of potassium hydrate. Specimens of this sort are beautiful and instructive: a black vascular system shows through a translucent embryo. These specimens proved to be a most valuable control in the study of the sections of the human embryos, for I had them in abundance, and I also got some ideas of the variations of the blood-vessels and their general relation to the surrounding structures.

ARTERIES.

It has been shown during recent years that in the embryo a series of segmental arteries arise from the aorta, which in the head-end of higher vertebrates unite on their distal ends to produce the two vertebral arteries. These in turn unite at the middle line to produce the basilar artery, as has been shown by His in his monograph on the human embryo. By this process of loop-throwing we have produced in the human embryo of four weeks two vertebral arteries which unite to form the basilar and on the anterior end join with the internal carotids. So as soon as the vertebral arteries unite to form the basilar we have marked off the circle of Willis, and considering its relation to the neural tube we can identify its branches to the brain as they arise. In the specimen four weeks old (Fig. 3) the arteries are not well marked, and it is difficult to outline the primary circle of Willis, let alone the branches arising from it. A specimen a little older has in it all of the circle of Willis with the primary arteries to the brain beautifully outlined (Fig. 4), and it is possible to follow them through the capillaries over to the veins. Were it not for the great number of variations found in the arrangement of blood-vessels it would be easy to identify most of the arteries in this specimen by considering them in relation to the cranial nerves and other structures.

The circle of Willis is fully formed in this specimen and extends from the bifurcation of the basilar artery to the anterior communicating. At the point the carotid enters the circle there is a short ophthalmic which is also present in the embryo of the fourth week (No. 2), and is shown in No. 74, Fig. 5. Throughout the region of the brain branches rise at quite regular intervals from the anterior communicating to the vertebral arteries. So regular are these branches that they might be spoken of as the segmental arteries to the brain. These are then gradually shifted, some becoming enlarged and others disappear.

The anterior and middle cerebral arteries (Fig. 4) arise as a common stem and form a main branch encircling the optic stalk from which small branches pass on the lateral side of the cerebral vesicle while the main stem continues to the front of the brain and communicates with its fellow on the opposite side immediately behind the olfactory pit. It is easy to imagine the anterior cerebral pushed into place when the cerebral vesicle protrudes over it in every direction. In embryo No. 74 (Fig. 5) the middle cerebral is much better marked, while the anterior cerebral cannot be followed to its end. In embryo No. 145 (Figs. 6-8) the adult form of these two vessels is well given. Numerous radiating branches mark the middle cerebral over the embryonic island (Fig. 6) and the anterior cerebral extends to the mesial side of the hemisphere as it does in the adult (Fig. 7). The anterior cerebral artery is pictured by His in his last great monograph on the brain² in an embryo³ of about the same age as my embryo No. 145. This illustration is from a sagittal series like the one from which my Figs. 6-8 were reconstructed. In His' paper this vessel is called "die vorder Bogenvene die das Blut aus dem vordern Abschnitte der Hemisphärenwand sammelt."⁴ When the direction of this vessel is considered, and especially when it is reconstructed, it is easily shown that His was in error in calling it a vein.

The anterior choroidal artery is next in order, for it also arises from the carotid artery and its destination, the choroid plexus, is well marked in young embryos. In Fig. 4, the artery which takes this position is intimately associated with the middle cerebral and lies between the cerebral hemisphere and the optic thalamus. It may be that what I have termed the anterior choroidal is in reality the middle cerebral, and that the artery more dorsalwards is in reality the choroidal, for it is well known that arteries often shift a great deal in young embryos. Not

² His: *Die Entwicklung des menschl. Gehirns.* Leipzig, 1904.

³ Page 79, Fig. 51.

⁴ P. 125

until their walls are fairly well developed are the arteries well fixed. The arrangement shown in Fig. 4 is again present in embryo No. 74, Fig. 5, and here the choroid plexus is well developed. In an older embryo, Fig. 8, the anterior choroidal artery is well marked, and it arises farther back,—from the posterior communicating artery. For this reason I often thought that the more dorsal artery in Fig. 4 represented the choroidal, and only in a much later stage than that shown in Fig. 8 is the anterior choroidal shifted from the posterior communicating to the carotid. To test this question further I examined numerous transparent pigs of corresponding stages in which the arteries only had been injected, and in all cases the anterior choroidal arose in common with the middle cerebral, and after this I was strongly inclined to consider the origin of the anterior choroidal from the posterior communicating in Fig. 8 as a variation.

The posterior cerebral artery is relatively late to develop, and in early embryos its place of origin is taken by a number of large branches to the mid-brain. This is very marked in Figs. 1, and 4 to 8. In the large embryo (Fig. 1) the vessels have been injected and in drawing it the cerebrum was pulled forward to show the large artery to the mid-brain. There is also a small posterior cerebral artery present showing that for a long time the artery to the mid-brain is much more prominent than the posterior cerebral. In the adult the posterior cerebral arteries mark the terminations of the basilar and lie immediately in front of the third and fourth cranial nerves. The arteries which fulfill these requirements supply the dorsal portion of the mid-brain, corresponding to the posterior quadrigeminal body in the adult brain. But in the adult the posterior cerebral artery, in addition to its main branches to the cerebrum, supplies much more than this, for it also sends branches to the crus, posterior part of the thalamus walls of the third ventricle, as well as elsewhere. In fact, all of the branches together arising from the circle of Willis between the third and fourth nerves behind and the origin of the middle cerebral in front (compare Figs. 4 and 9) must become united to produce the posterior cerebral artery. The region supplied by these numerous branches in the embryo is supplied by the posterior cerebral in the adult, and in its development these branches must be gradually drawn together into one stem to produce the final condition. And this is to be expected. It is only after the cerebrum makes its appearance and reaches the great prominence it does in man that the condition found in the lower vertebrates is overshadowed. At a relatively late stage, later than the one shown in Fig. 1, all of these arteries arise from a single trunk. In this there are still two main trunks which must unite subsequently to form the posterior cerebral.

The posterior communicating branch must be formed by shifting nearly all of its branches, as shown in Fig. 4, back to the third and fourth nerves to produce the posterior cerebral artery, while the cerebral hemisphere is growing over the thalamus and mid-brain. Or by a series of arches, as indicated in Fig. 4, the arteries to the more dorsal portions of the thalamus and mid-brain, as well as to the structures which wander into this region, are gradually transferred towards the basilar, leaving the small branches of the posterior communicating to supply the immediate neighborhood, as it does in the adult. At any rate the large vessel arising from the posterior communicating in Fig. 1 arises from the posterior cerebral, probably as the postero-lateral set, in the adult. The question is further complicated by variations, which are quite numerous, the most common variation of the posterior cerebral being in its origin, which is transferred from the basilar to the internal carotid.⁵ I have also found all kinds of combinations of this artery with neighboring arteries in the embryo pig, which I interpret in part as transformation stages. Furthermore, it may be possible that shifting of arteries takes place until the individual is fully grown, for Bean⁶ has shown recently that the branches of the subclavian artery of the infant differs from those of the adult.

The branches from the basilar and vertebral arteries are more easily followed, for in this region there is less shifting, and the landmarks prove to be of more value. In the upper part of the mid-brain there is a cluster of branches which are destined to become the superior cerebellar arteries (Fig. 4). This group is reduced to a single artery in Figs. 5 and 6, where it is just behind the isthmus. The next group in Fig. 4 is the transverse pontine between the superior cerebellar and the otic vesicle. Then the anterior cerebellar between the seventh and eighth nerves near the otic vesicle. Finally, the group which perforates the root of the twelfth nerve is destined to form the posterior inferior cerebellar. This branch is also shown in Fig. 1. Between this and the otic vesicle there are a couple of branches, shown in Fig. 4, the fate of which is uncertain.

VEINS.

While the arteries of the brain undergo many changes in their development, their history is relatively simple when compared with the gyra-

⁵ According to Windle (*Journal of Anatomy and Physiology*, XXII, 1888), this variation occurred 28 times in 400, or in 7 per cent of the cases.

⁶ Bean: *American Journal of Anatomy*, Vol. 4.

tions the veins undergo. The subject is, however, simplified to a great extent by the excellent studies of Hochstetter and his pupils, Salzer, and Groszer and Brezina, who have unraveled many of the tangles of the anterior cardinal vein while it is being transformed into the brain sinuses. The study of the development of the veins of the head of the guinea-pig by Salzer⁷ is especially of value to me, for it takes up a number of points which would be difficult to interpret properly from my material.

It is generally believed since the time of Luschka⁸ that the blood from the veins of the brain leaves the embryonic skull through a foramen in front of the temporal bone—the foramen jugulare spurium—and empties into the external jugular vein. A secondary communication is formed with the internal jugular vein, which in man and in monkeys is the only outlet of the brain sinuses, both communications remaining open to a greater or less degree in many vertebrates. Luschka also found a human skull with a foramen jugulare spurium present between the temporal bone and the glenoid fossa. This opening is referred to frequently in the various text-books on anatomy,⁹ and it is explained by stating that it is the remains of a channel through which the blood poured in the foetus. This explanation may be correct as far as it goes, but when it is asserted that the brain sinuses at first communicate with the external jugular vein through the foramen spurium and later with the internal jugular vein, a conclusion is drawn which the facts do not warrant. Although Salzer showed conclusively that the internal jugular vein receives all the blood from the brain from the very earliest stage, and that the connection with the external jugular is of much later formation, Luschka's statement is still retained in the text-books.¹⁰ While Salzer corrected the erroneous interpretation of Luschka, he also discovered that in the embryo the veins first leave the embryonic skull through a canal near the seventh nerve, and then emptied into the internal jugular vein. All this takes place long before there is a trace of an external jugular vein present, so the idea of Luschka that the external jugular vein is the primary vein for the blood from the brain is untenable, and should be removed from the text-books as soon as possible.

Salzer's work shows that the anterior cardinal veins of mammals are placed on either side of the chorda ventral to the brain between the

⁷ Salzer: *Morph. Jahrbuch*, XXIII, 1895.

⁸ Luschka: *Denkschriften der Wiener Akademie*, XX, 1862.

⁹ See for instance Cunningham's Anatomy, p. 116.

¹⁰ Cunningham's Anatomy, Figs. 603 to 606.

roots of the cranial veins. They soon begin to shift lateralwards, and by a process of sprouts encircle the cranial nerves successively and soon come to lie to the lateral sides of the nerves. In other words, the nerve trunks have wandered through the veins and changed positions with them. This all takes place without interrupting the circulation through the vein. In Fig. 3 the vein is shown partly wandered out, the otic vesicle, seventh and ninth nerves now being on the medial side of the vein. A number of sprouts encircles the tenth nerve, which in Fig. 9 is also on the medial side of the vein. Finally the fifth nerve wanders through the vein. In Fig. 13 this has taken place in part, and in Fig. 11 the change in position is complete. What I have here given in rapid order comprises the results found by Salzer in the guinea-pig, and I repeat it only to illustrate his point with my figures. Now Salzer calls the vein which lies to the medial side of the nerves the anterior cardinal vein, and that portion which moves to the lateral side of the nerves the vena capatis lateralis.¹¹ After the vein crosses the twelfth nerve passing to the heart he calls it the internal jugular. These terms I shall retain, for they are most useful in the description of the fate of the anterior cardinal vein. So findet wir, says Salzer (*l. c.*, p. 248), bei den untersuchten Säugetieren sehr schön übereinstimmende Verhältnisse in Bezug auf die erste Entwicklung der Venen des Kopfes. Ueberall wird die ursprünglich medial von den Kopfnerven gelegene Vene durch ein Gefäß ersetzt, das eine laterale Lage den Nerven gegenüber einnimmt. Diese Lageveränderung geht durch Inselbildung vor sich, und zwar bilden sich derartige Inseln zuerst um Acustico-facialis, fast zu gleicher Zeit auch um den Vagus herum, dann erst erfolgt die Verlagerung der Venenbahn dem Hypoglossus gegenüber: dem Trigeminus gegenüber behält die Vena verhältnismässig lange ihre ursprüngliche Lage bei. Ist das knorpelige Skelet angelegt so verlässt das Blut der vorderen Hirnabschnitte gemeinsam mit dem Facialis die Schädelhöhle, während das Blut des Hinter- und Nachhirns von einer Vene gesammelt wird, die durch das Foramen jugulare an der lateralen Seite des Vagus nach auszen zieht: hier verbinden sich beide Gefäße zur Vena jugularis interna. Bald jedoch obliterirt nach Ausbildung einer Anastomose dorsalwärts vom Gehörorgan die neben dem Facialis austretende Vene, so dass die neben dem Vagus austretende Vene die einzige abführende Blutbahn des Schädels darstellt. An dieses Verhalten schlieszen sich die nun sekundär auftretenden Verbindungen der Gefäße des Schädelinneren theils mit den Gesichtsvenen, theils mit den Venen des Rück-

¹¹ His (*Entwickel. des menschl. Gehirns*) calls this vein Basalvene.

enmarkes an. Dabei geht die Bahn durch das Foramen jugulare entweder vollständig oder zum Theil zu Grunde. Die bei den meisten Säufern auftretende sekundäre Verbindung ist die, welche das Foramen jugulare spurium zum Austritte benutzt, doch giebt es auch Thierformen, z. B. die Katze, bei denen ein solches gar nicht zur Ausbildung kommt, obwohl die Vena jugularis interna fast vollständig zu Grunde gegangen ist. Hier treten eben die sekundären Verbindungen, welche die Orbital- und Nachhirnvenen eingehen, für diese Gefasze ein. Mithin kann man wohl behaupten, dass die Vena jugularis interna als Fortsetzung des Sinus transversus, wie sie beim Menschen und beim Affen am schönsten ausgebildet ist, ein primitiveres Verhalten darstellt, bei welchen die Vena jugularis externa die hauptsächliche, wenn nicht die einzige abführende Bahn des Schädelinneren darstellt.

It is apparent from the above that the beginning of the internal jugular vein is marked by the twelfth nerve crossing the anterior cardinal vein and that it extends to the lateral sinus through a number of sprouts behind the otic vesicle (Fig. 13). The vena capatis lateralis is that portion of the anterior cardinal vein which wanders to the lateral side of the cranial nerves and extends from the twelfth to the fifth nerve. The portion of the anterior cardinal vein which lies medial to the fifth nerve retains that position throughout its development and marks the cavernous sinus (Fig. 10). Into the embryonic cavernous sinus there empties the ophthalmic and anterior cerebral veins. The latter soon extend to the embryonic superior sagittal sinus. Between the fifth and 7th nerves a vein extends to the region of the cerebellum, the vena cerebralis media (Figs. 9, 10 and 13), and behind the otic vesicle there extends through the embryonic jugular foramen the vena cerebralis posterior (also shown in Fig. 11).

We have, therefore, in the embryos of the second month an arrangement of the veins in the head similar to that found in the reptiles as shown by Groszer and Brezina,¹² and I shall employ the nomenclature of these authors. The anterior cardinal vein shifts lateralwards and by the end of the first month is partly lateral to the otic vesicle as shown in Fig. 3. The process is shown more advanced in Fig. 9, and is complete in Fig. 13. The condition shown in Fig. 13 is that which is permanent in selachians. Now the reptilian stage is entered. From the lateral vein—the vena capatis lateralis—three veins extend into the head and encircle the brain. The first—the vena cerebralis anterior (shown well in Fig. 10)—passes up and over the cerebral vesicle, *i. e.*,

¹² Groszer and Brezina: Morph. Jahrb., XXIII, 1895.

the region of the island. The second—the *vena cerebralis media* (Figs. 9, 10 and 13)—arises at the anterior juncture of the *vena capatis lateralis* with the anterior cardinal vein between the fifth and seventh nerves. The third—the *vena cerebralis posterior*—arises from the posterior part of the *vena capatis lateralis* with the internal jugular behind the otic vesicle and enters the embryonic skull through the jugular foramen (Fig. 12), and ultimately becomes the transverse sinus. From this simple reptilian stage the mammalian is formed, and in man but little must be added to, and but little subtracted from, the general plan.

The anterior end of the anterior cardinal vein remains in large part on the medial side of the fifth nerve in the human embryo and is ultimately transformed into the *cavernous sinus*. From the earliest stages the *ophthalmic vein* enters this sinus as is shown in all of the embryos studied. Although I have no evidence regarding the development of *intercavernous sinus*, it is easy to understand its development by branches from the cavernous sinus growing to encircle the hypophysis, and then to unite, thus forming a plexus around it. So also by an extension of the cavernous sinus forwards the *spheno-parietal sinus* must be formed. In the early embryos the anterior cardinal vein or the portion which forms the cavernous sinus is extended forward to form the *vena cerebralis anterior*, which ends in the bilateral superior sagittal sinus as shown in Fig. 3. With this the veins from the region of the island communicate as shown in Fig. 10, the basal portions of which are evidently retained to form the *middle cerebral* (superficial Sylvian) *vein*. So also the superior sagittal sinus, the superior and inferior petrosal sinuses and the *vena capatis lateralis* are directly continuous with the cavernous sinus from their beginning.

I have spoken enough about the *vena capatis lateralis* above, and wish only to add its relation to the permanent brain sinuses in man at this point. It may be defined as that portion of the anterior cardinal vein which is transferred to the lateral sides of the cranial nerves extending in the human embryo from the fifth to the twelfth cranial nerves, being directly continuous in front with the anterior cardinal vein, or better the cavernous sinus, and behind with the internal jugular vein. This vein is clearly outside of the skull, leaving it between the fifth and seventh nerves (Fig. 12), and then communicating with the internal jugular. It is this vein which Kölliker believed to be the external jugular, and apparently confirmed Luschka's notion regarding the relation of the external jugular vein to the brain sinuses. It certainly does leave the skull along the root of the seventh nerve, a line in common with the so-called foramen jugulare spurium, but it disappears long before

external jugular vein is formed, as shown by Salzer. The vena capatis lateralis is fully developed during the fifth week, as is best shown in Fig. 13. In this embryo it is irregular in shape, ending in a lakelet behind, a condition which may also be due to the way the blood accumulated in this vein just before the death of the embryo. In Fig. 10, which is from an embryo in which these veins were gorged with blood, the lakelet is not present. Soon the vena capatis disappears, and veins more dorsalwards carry blood from the brain, as shown in Fig. 11.

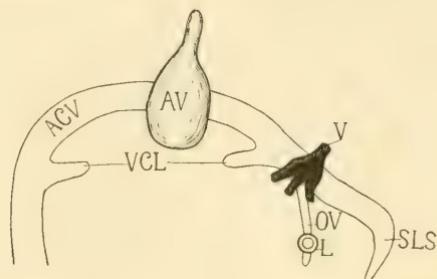


FIG. 14.

FIG. 14. Diagram of the veins of the head of an embryo four weeks old. *ACV*, anterior cardinal vein; *VCL*, vena capatis lateralis; *SLS*, superior sagittal sinus; *AV*, auditory vesicle; *V*, fifth nerve; *L*, eye.

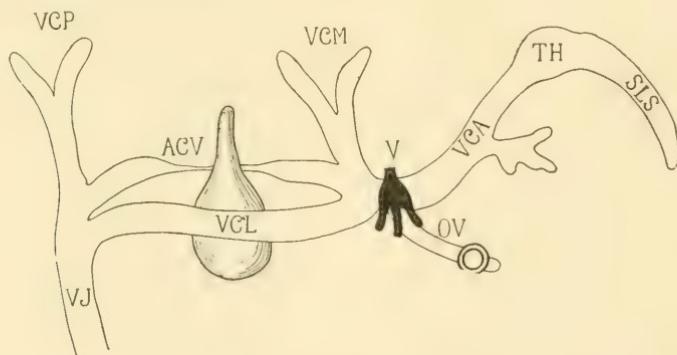


FIG. 15.

FIG. 15. Diagram of the veins of the head during the fifth week. *VCP*, vena cerebralis posterior; *VCM*, vena cerebralis media; *VCA*, vena cerebralis anterior; *TH*, torcular Herophili; *OV*, ophthalmic veins; *VJ*, jugular vein.

When the vena capatis is well developed it sends from its two extremities two main veins to encircle the brain and to collect its blood. The first of them, the *vena cerebralis media*, arises at the point of juncture between the vena capatis lateralis and the cavernous sinus and extends between the fifth and seventh nerves towards the region of the cerebellum.

The *vena cerebralis posterior* arises from the *vena capitis lateralis* more dorsalwards, at its juncture with the *vena jugularis interna*, and encircles the hind-brain in the region of the twelfth nerve. These two veins are well shown in Figs. 9 and 13.

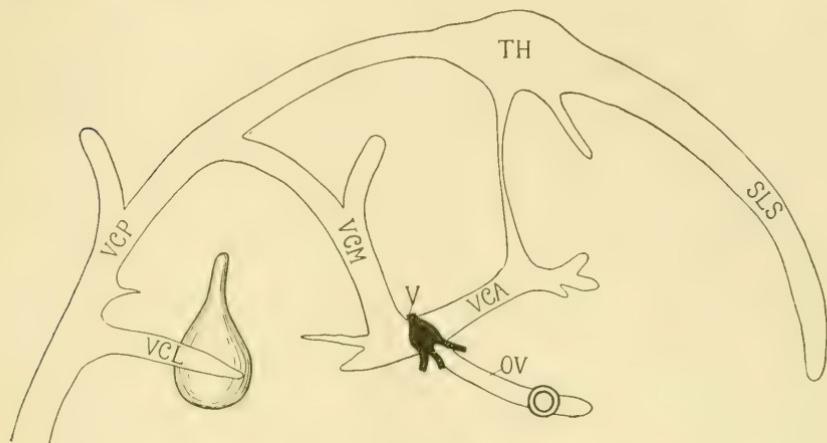


FIG. 16.

FIG. 16. Diagram of the veins of the head at the beginning of the third month. Lettering as before.

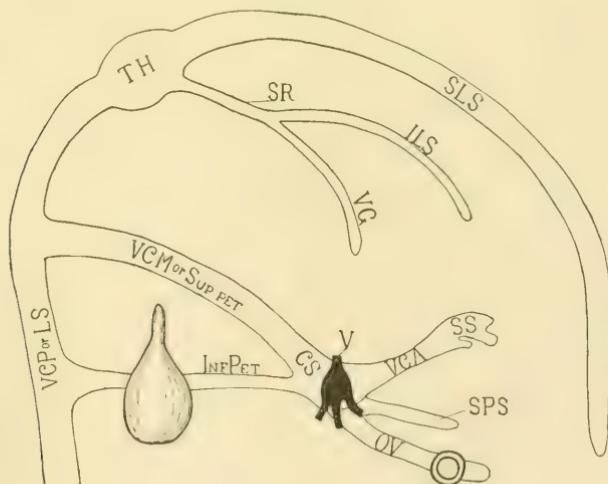


FIG. 17.

FIG. 17. Diagram of the veins of the brain of an older foetus. Lettering as before. LS, transverse sinus; SR, sinus rectus; ILS, inferior sagittal sinus; VG, great cerebral vein (Galen); CS, cavernous sinus; SS, middle cerebral vein (Sylvian); SPS, spheno-parietal sinus; Sup. Pet., superior petrosal; Inf. Pct., inferior petrosal sinus.

The *superior sagittal sinus* is formed by an accumulation of small veins over the dorsal side of the cerebral vesicle (Fig. 3), which sometimes appear as a tuft (Fig. 13) and at other times as a lakelet (Fig. 9). Soon the sinuses of the two sides communicate (Fig. 10), and from now on the paired sinuses are single. At first the sinuses communicate with the anterior cardinal vein (the cavernous sinus) through the vena cerebralis anterior, and these two veins take up all of the small veins from the cerebral vesicle (Fig. 10). With the growth of the cerebrum the superior sagittal sinus is shifted downwards and its communication with the cavernous is broken. It now communicates with the vena capatis lateralis through the vena cerebralis media, a transitional form being shown in Fig. 10. With great rapidity the communication is transferred to the jugular through the vena cerebralis posterior, which leaves the skull through the jugular foramen. This stage is shown in part in Fig. 11, which is a reconstruction from a partial natural injection. However, even in this case the superior sagittal sinus must be shifted more dorsalwards, for in this embryo it still passes lateral to the otic vesicle, and therefore in this region it is outside of the skull. The same criticism can be made of Salzer's figure of the corresponding stage in the guinea-pig.¹³ Here also the vena capatis lateralis, as well as the first dorsal anastomosis, is lateral to the otic vesicle, and therefore cannot possibly be the permanent vein in this animal. In order to reach the permanent form, as shown in Salzer's Fig. 5, a second dorsal anastomosis must be established, and this is well begun, as Salzer's Fig. 4 shows. So in order to complete the superior sagittal and transverse sinuses a more dorsal anastomosis must be established than that shown in Fig. 11, and the indications for this are present in this figure, as well as in Fig. 10. In this latter figure the superior sagittal sinus must be transferred completely from the vena cerebralis anterior to the vena cerebralis posterior, and in so doing the vena capatis lateralis is obliterated. In case they all remained open, we would have the condition found in *Tropidonotus*,¹⁴ but this is not the case, as is indicated in Fig. 11. The complete condition of the superior sagittal sinus is shown in Fig. 8. Here the internal jugular communicates through the vena cerebralis posterior with the dorsal end of the superior sagittal sinus along the line of the hind-brain and mid-brain. The steps towards this are all indicated in Fig. 10. Therefore the main portion of the *transverse sinus* is formed directly from the vena cerebralis posterior.

¹³ Salzer: Morph. Jahr., XIII, Taf. XVIII, Fig. 4.

¹⁴ Groszer and Brezina: Morph. Jahr., XXIII, Taf. XXI.

If now the vena cerebralis media, as shown in the human embryo, is compared with that in *Tropidonotus*, and in turn with that of the adult sinuses, it is seen that the vena cerebralis media is the *superior petrosal sinus*. They all communicate with the cavernous sinus between the fifth and seventh nerves, they lie lateral to the cranial nerves behind the fifth, and they are also medial to the otic vesicle, *i. e.*, they are within the skull. This latter condition is not yet the case in Fig. 11, but is indicated in Fig. 10, and it is marked by the stub vein near the pons in Fig. 8.

The dilatation at the posterior end of the superior longitudinal sinus marks the beginning of the *torcular Herophili* (Fig. 8), and from it the *sinus rectus* extends towards the choroid plexus, where it ends in the *great cerebral vein*. Between the straight sinus and the superior sagittal sinus a small vein enters the falk and ends at once in a capillary plexus. This vein no doubt marks the beginning of the *inferior sagittal sinus*. Behind the transverse sinus (Fig. 8) there is a second venous anastomosis extending from the region of the mid-brain to the internal jugular vein, and no doubt marks the *occipital sinus*, which in the adult is as an anastomosing channel between the upper and lower ends of the transverse sinus. Extending forwards from the juncture of the transverse sinus with the internal jugular vein, a venous sprout is shown in Fig. 8, which passes on the outside of the skull towards the seventh nerve, and marks the remnant of the *vena capatis lateralis*.

In the youngest human embryos the anterior cardinal veins run on the medial side of all of the cranial nerves before the *vena capatis lateralis* is formed. In case the whole of the anterior cardinal vein remained permanently, that portion between the cavernous sinus and the internal jugular vein would become the *inferior petrosal sinus*, for they both hold the same position. But it appears that the inferior petrosal sinus is of new formation, for in none of the intermediate stages can a trace of it be found.

A résumé of the development of the sinuses of the brain from the anterior cardinal vein is given in Figs. 14 to 17. They explain themselves.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1. Surface of the brain with the arteries injected in an embryo 48 mm. long (No. 237). Enlarged 5 times; injected by Mr. Brödel. The dorsal end of the cerebral vesicle has been drawn forward to show better the vessels of the mid-brain.

FIG. 2. Surface view of the brain of an embryo 80 mm. long (No. 234a). Slightly enlarged from a photograph by Dr. Mellus. Over the region of the

island the extravasation is extensive, while over the rest of the brain it is in spots along the arterioles, as they penetrate the brain.

FIG. 3. Embryo of the fourth week (No. 2). Enlarged 16 times. The external form is from nature. The structures within the head have been reconstructed: the tenth and twelfth cranial nerves and the first cervical nerve by Dr. Streeter.

FIG. 4. Brain and its arteries of embryo No. 163. Enlarged 15 times. The picture of the brain is from a wax-plate model by Dr. Lewis. The arteries are from a graphic reconstruction. The basilar artery extends throughout the length of the hind-brain and the circle of Willis throughout that of the fore-brain. The position of the cranial nerves and otic vesicle is given in Fig. 9.

PLATE II.

FIG. 5. Graphic reconstruction of the head of embryo No. 75, showing the arteries and the brain. Enlarged 7 times.

FIG. 6. Graphic reconstruction of the brain and arteries of embryo No. 145. Enlarged 7 times.

FIG. 7. Same as Fig. 6. The right cerebral hemisphere has been removed, showing the anterior cerebral artery throughout its extent.

FIG. 8. Same as Fig. 7 with the choroid plexus and large veins added.

FIG. 9. Embryo No. 163. Enlarged 13 times. The surface view is from an excellent photograph, and the structures in the head are from a graphic reconstruction.

PLATE III.

FIG. 10. Graphic reconstruction of the veins and brain of embryo No. 74. Enlarged about 10 times.

FIG. 11. Graphic reconstruction of veins of the head and brain of embryo No. 144. Enlarged about 7 times.

FIG. 12. Section through the head of embryo No. 109. Enlarged 12½ times. *H*, hypophysis; *S. Ob*, superior oblique muscle; *Ex. R*, lateral oblique muscle; *Tr*, trapezius; *VJ*, jugular vein. The cranial nerves are numbered with Roman numerals. On the side lettered the section is nearer the mouth than on the other side, showing that the vena capitis lateralis which connects with the jugular vein is on the outside of the skull.

FIG. 13. Head of embryo No. 109. Enlarged 12½ times. The form of the arm and body are from photographs. The face, brain and nerves are from a wax-plate reconstruction by Dr. Lewis. The veins are from a graphic reconstruction. The brain and face are somewhat distorted, but are given in this way to complete Fig. 5, Plate IV, in the publication of Bardeen and Lewis in vol. I of this journal.

DEVELOPMENT OF THE BLOOD-VESSELS OF THE HUMAN BRAIN
FRANKLIN P. MALL

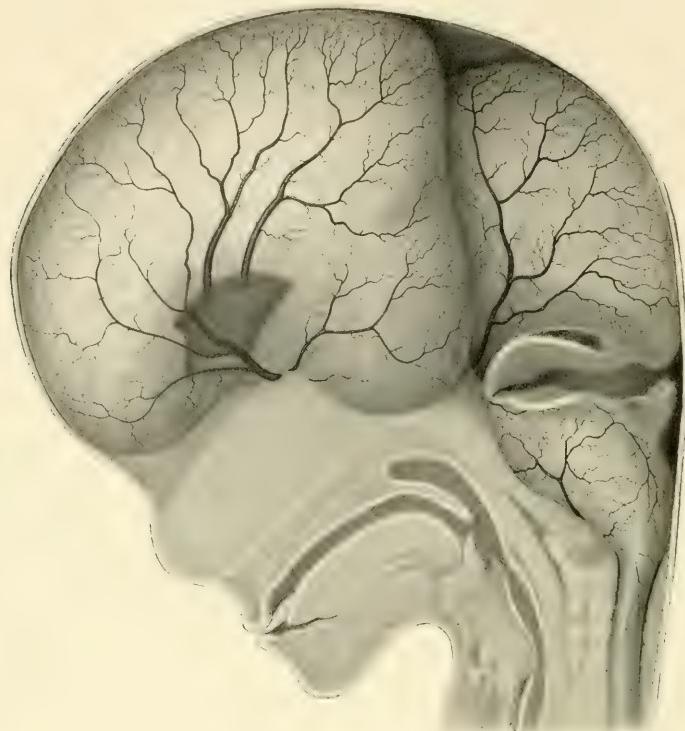


FIG. 1.



FIG. 2.

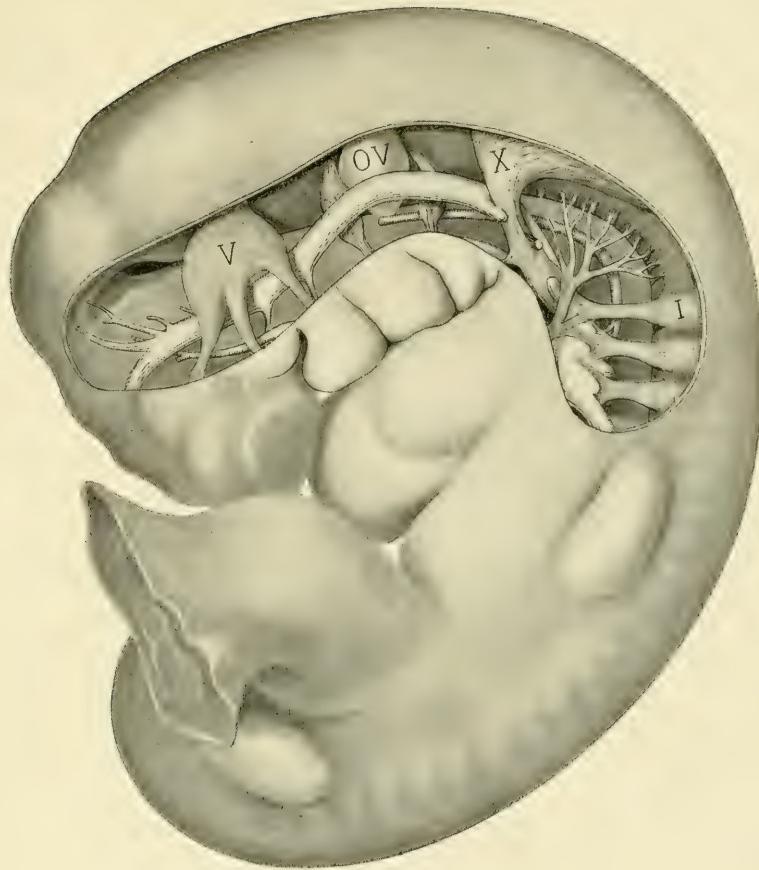


FIG. 3.

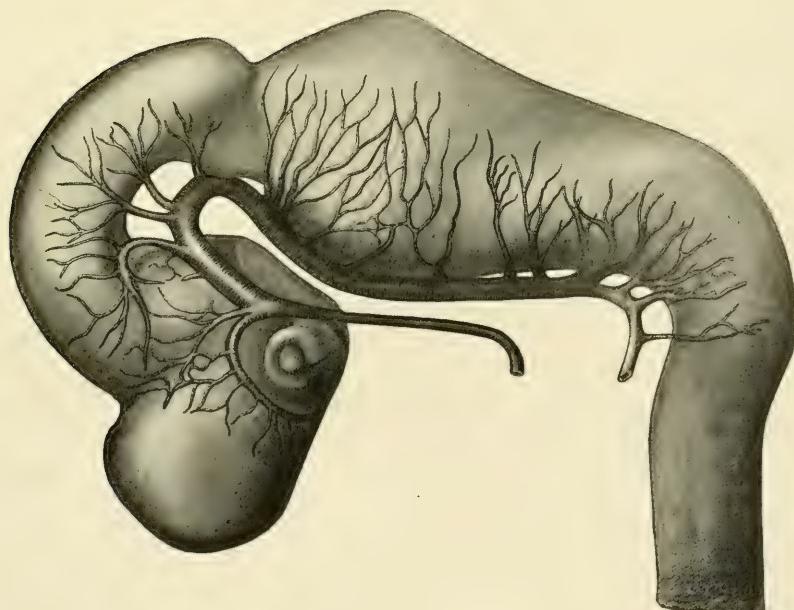


FIG. 4.

Kline, del.

DEVELOPMENT OF THE BLOOD-VESSELS OF THE HUMAN BRAIN
FRANKLIN P. MALL

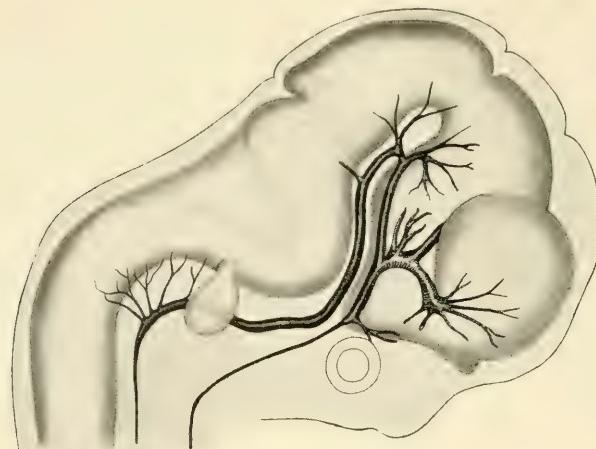


FIG. 5.

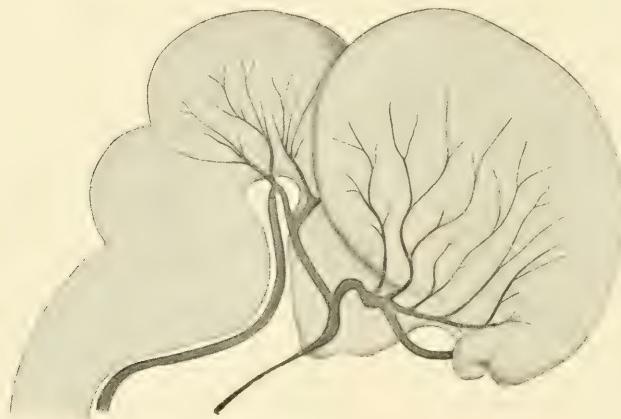


FIG. 6.

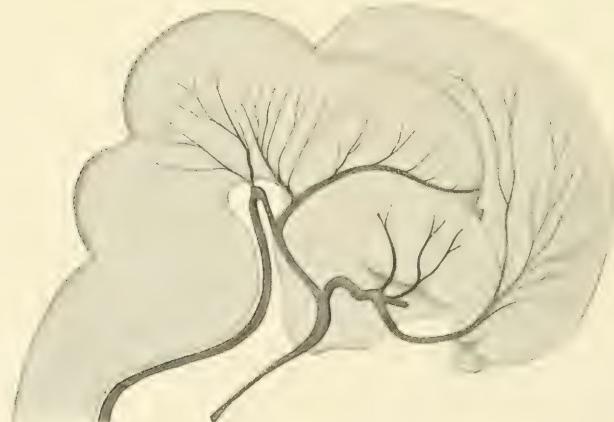


FIG. 7.

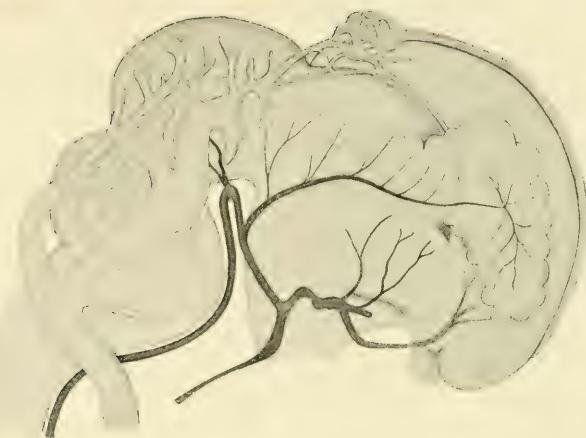


FIG. 8.

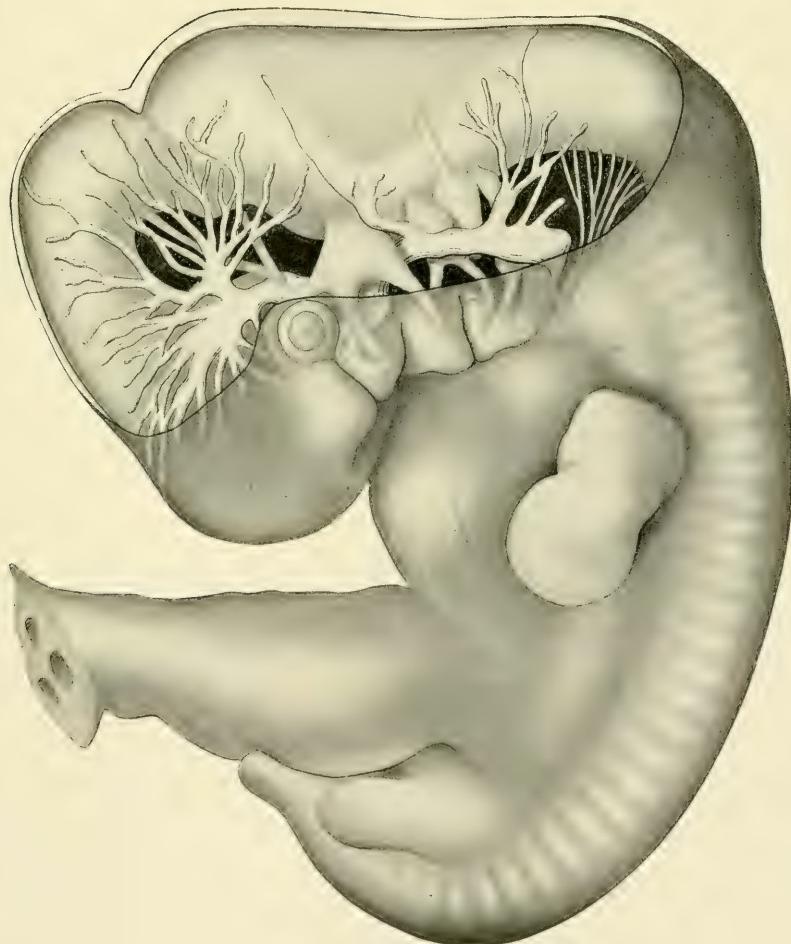


FIG. 9.

Kline, del.

DEVELOPMENT OF THE BLOOD-VESSELS OF THE HUMAN BRAIN
FRANKLIN P. MALL

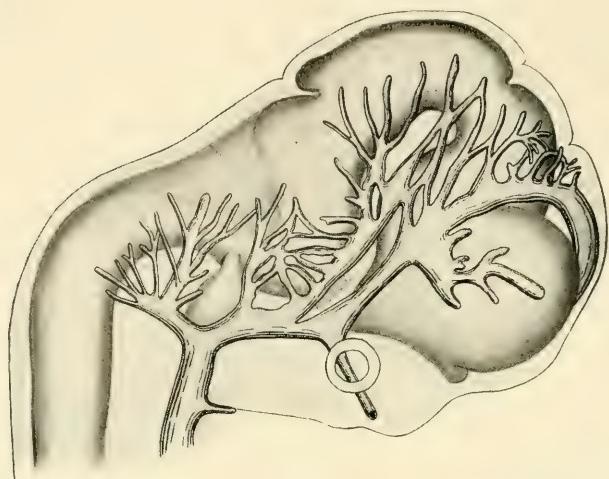


FIG. 10.

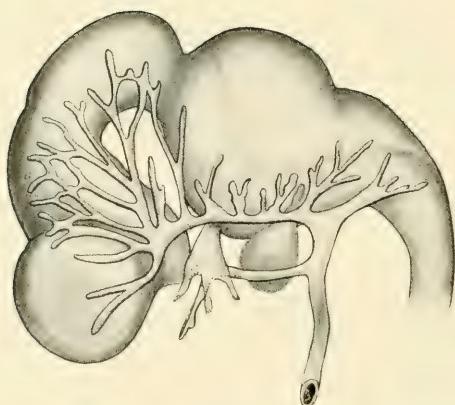


FIG. 11.

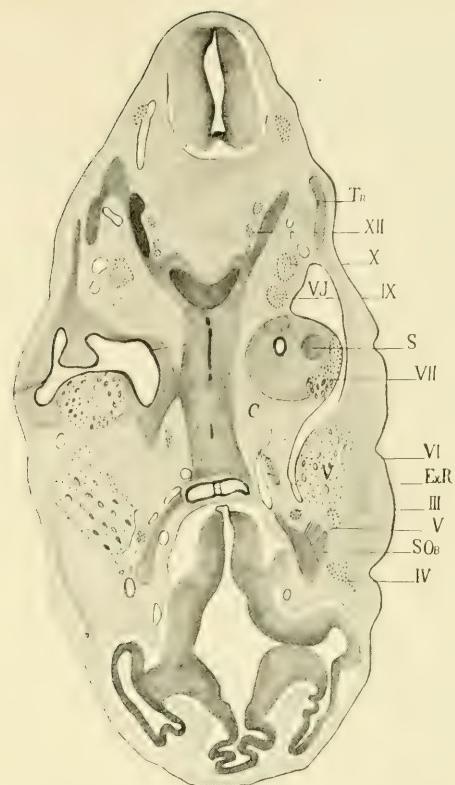


FIG. 12.

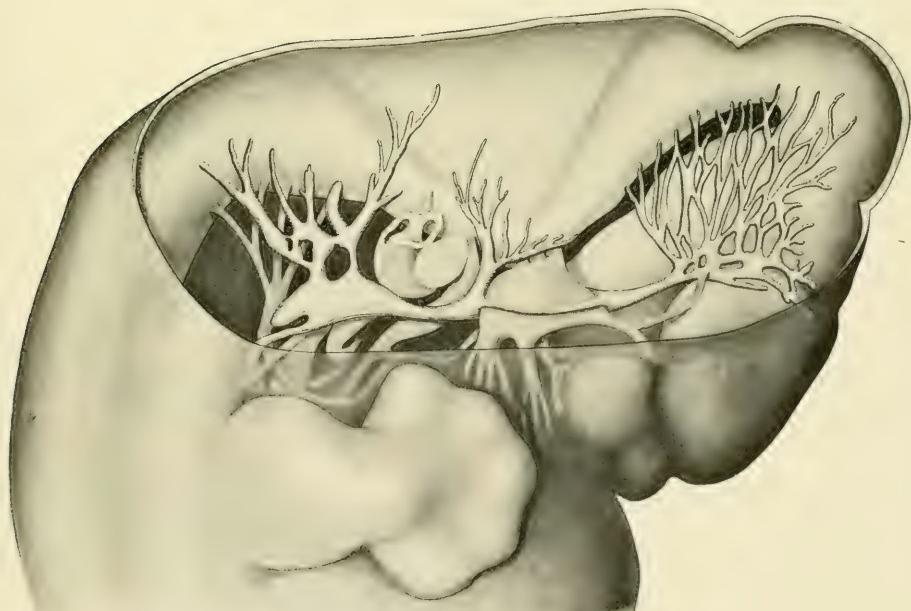


FIG. 13.

Kline, del.

THE SIZE OF THE ARTICULAR SURFACES OF THE LONG
BONES AS CHARACTERISTIC OF SEX; AN
ANTHROPOLOGICAL STUDY.

BY

THOMAS DWIGHT, M. D., LL. D.,

Parkman Professor of Anatomy at the Harvard Medical School.

WITH 6 PLATES.

The pelvis has long been recognized as a reliable guide to the sex of the skeleton and still longer as the greatest peculiarity of the female figure. From twenty to thirty years ago several papers appeared on the means of determining the sex of the skull. It is, I think, now generally admitted that the skull is of value in the hands of an expert; but the late Professor Brinton very near the end of his life declared that apart from the pelvis there is no guide to the sex among the bones.

Hyrtl (1) long ago wrote: "I find the difference between the male and female sternum so clearly expressed by the proportion of the manubrium to the body that it is hardly possible to err in determining the sex. The manubrium of the female sternum exceeds in length that of half the body; while in the male sternum it is at least twice as long as the manubrium. I (2) was able to show on sufficiently large series that while this was true of the average male and female sterna, it was not true of about 40 per cent of the individual instances, so that it was very possible indeed to err in determining the sex by that means. Probably the rule applies to well-formed bodies, but not to a large proportion of those that we meet with. The femur again is a bone that is to the expert of much value. A typical male and a typical female femur can hardly be mistaken; but practically there are a great many thigh bones, perhaps 75 per cent, on which an expert would be unwilling to give an opinion by methods hitherto in use. Without going so far as Professor Brinton, we may say that with our present methods, excepting the pelvis, and even this is not always conclusive, in the great majority of cases the expert must form his opinion of the sex of bones from their general appearance, and that comparatively rarely can he speak (still excluding the pelvis) with any great certainty."

The purpose of this paper is to present a new method, which indeed I have suggested before, but which I had not established by a sufficient series of observations; namely, the relatively small size of the articular surfaces of the long bones in the female. If this be true it certainly deserves a place among the laws of anthropology. While I believe that this applies to the long bones in general, I have limited the demonstration of the principle to the heads of the humerus and femur.

In the Shattuck lecture on the *Range and Significance of Variation in the Human Skeleton*, which I had the honor of giving before the Massachusetts Medical Society in 1894 (3), I advanced the opinion that the size of the articular surfaces of the limbs has an important sexual significance. I mentioned that I had studied the dimensions and proportions of the glenoid cavity of the scapula on 63 male and 27 female bones. Its average length in the male bones was 3.92 cm. and in those of the female 3.36 cm. Very few male ones were less than 3.6 cm. and very few female as long. Though I had made observations on the bones of the arm and forearm I had no series large enough to quote; but I spoke more in detail of the observations on 64 femora on which many measurements had been taken, and which came from bodies that had been measured before dissection. After discussing some of the more common features as guides to determine the sex, I said: "Some other measurements seem to throw more light on this matter. They tend to establish the theory that the small size of joints is characteristic of woman. They are the greatest diameter of the head of the femur and the greatest transverse breadth through the condyles. The average diameter of the male head is 4.8 cm., that of the female 4.15. My tables show one marked difference between the sexes; namely, that in the women there is a fairly regular increase in the size of the head corresponding with the increase in length of the femur. Among men this is not so. While it is true that most of the largest heads are found in the longer half of the bones and most of the smallest in the shorter half, the correspondence is far less evident. I find, moreover, that but two male heads have a diameter of less than 4.5 cm., and but two of the female a greater. Both these female bones were among the longest, but the two male were but little below the average. Thus it would seem that the actual measurement of the head of the femur is a pretty good criterion of the sex. The measurements of the knee are less conclusive. The average difference is just under one centimeter (8.3 and 7.3), but there are more that overlap."

Dr. Hepburn (4) published in 1896 measurements of femora of many races, one of the measurements being that of the head. He did not,

however, make any attempt to consider the sexual significance of the head. In fact, there were but few females among the bodies of Europeans from which the bones were taken. He mentions, however, incidentally, that the diameter of the head of the male femur was never below 40 mm., except among the Andamans; and also that a diameter below 40 was found in several female bones of various races.

Dr. Dorsey (5) published in 1897 a paper recording observations on the long bones of American aborigines in which he tested the accuracy of my views, taking the greatest diameter of the head of the humerus and of the femur and also the breadth of the upper end of the tibia. The sex was first decided from the pelvis. His results from the heads of the bones of 135 skeletons of various races of both North and South America were very strikingly in confirmation of the value of the size of the joints as a sexual characteristic. "Thus, if the maximum diameter of the head of the humerus of any American skeleton measure 44 mm., the chances are extremely great that it is a male; if it measure 45 mm., it is a male to a practical certainty. The inference to be drawn from the measurements of the femur seem almost, if not quite, equally valuable; and it would almost seem that we could determine the sex from the femur alone with a great deal more certainty than we could from the skull. After Professor Dwight's disparaging remarks about his results from measurements through the condyles of the femur, I was quite unprepared for the results which have been derived from the tibia. The range of variation is, to be sure, greater than it is for either the humerus or the femur; and this, it may be repeated, is largely due to the discrepancy in stature between the North and South American skeletons, but the dividing line for the two sexes, between 71 mm. and 72 mm., is almost as sharp as it is for the femur, and makes the tibia a valuable aid for the determination of sex."

Although I was satisfied that the principle that the small size of joints is characteristic of woman is correct, I felt that it should be established by a series of measurements large enough to be beyond question. Accordingly, I undertook to make the measurements of the head of one humerus and one femur of 100 male and as many female bodies. Those of white adults only were used. The head of the humerus was measured in both the vertical and the transverse diameter, the object being to get the greatest diameter for each, even if it deviated somewhat from the strictly vertical or transverse plane. In the femur the greatest possible diameter was carefully sought for. The measurements were made with blunt calipers. The bodies were those used for anatomy and surgery in the Harvard Medical School. I took the measurements when the cartilage was still

fresh. This is certainly proper for the purpose of an anthropological study as it represents the size of the joints as they are in life. Moreover, on many dried bones, the cartilage remains as a very thin layer, which, though amounting to little, causes a discrepancy between those bones and others in which it has been quite removed. The question of what deduction from diameters thus obtained should be made in comparing them with those from dry bones shall be considered later.

When I had obtained these measurements on 100 male and 100 female bones I tabulated the results and drew the curves. While the results seemed to establish the law, the curves were so irregular that it seemed certain that they could hardly show a true mean. I then made 50 more examinations in each sex, and again was dissatisfied with the curves, and undertook 50 more. Thus I have now the measurements of 400 bones equally divided between the sexes. Owing to the relative scarcity of female subjects, I think the additional hundred measurements of female bones has retarded me by nearly three years. The bones were those of white adults, and in every case the humerus and femur were both measured, so that comparisons can be made between the upper and lower extremity. It was not possible to restrict the measurements to bones of one side, as post-mortem injury or some pathological blemish often rendered at least one of the joints unavailable. They seem to establish the point at issue.

The averages are as follows:

	<i>Head of Humerus.</i>	<i>Head of Femur.</i>
Vertical.	Transverse.	
Male.....	48.76 mm.	49.68 mm.
Female	42.67 "	43.84 "
Difference.....	6.09 mm.	5.84 mm.

The above average measurements of the female are to the respective male ones as 87.51, 87.28; and 88.24 are to 100. (Plates I, II and III.)

It is easy to see by the curves (Plates I, II and III) that there is only 1 male with a vertical diameter of the head of the humerus below that of the average female, and only 2 females with the same diameter above that of the average male. Taking the transverse diameter of the head of the humerus we find 2 males below the female average, and 3 females above the male average. With the head of the femur we have but 1 male below the female average and but 1 female above the male average.

Taking separately the three series, each of 400 measurements, the following deductions may be made:

Head of Humerus, vertical diameter.

In the 36 smallest 0 male.	In the 51 largest 0 female.
" 94 " 1 "	" 85 " 1 "
" 133 " 4 "	" 111 " 2 "
" 171 " 9 "	" 135 " 3 "
	" 165 " 10 "

Head of Humerus, transverse diameter.

In the 55 smallest 0 male.	In the 42 largest 0 female.
" 94 " 2 "	" 69 " 1 "
" 126 " 3 "	" 107 " 3 "
" 155 " 7 "	" 139 " 5 "
	" 177 " 9 "

Head of Femur, greatest diameter.

In the 36 smallest 0 male.	In the 51 largest 0 female.
" 83 " 1 "	" 107 " 1 "
" 119 " 4 "	" 133 " 3 "
" 154 " 6 "	" 168 " 17 "

Continuing this line of comparison I was anxious to divide the bones into a smaller and a larger half, and to see how many male bones were among the 200 smaller and how many female among the 200 larger, taking each of these diameters successively. Unfortunately the groups did not allow this division to be made without putting some of a group of equals into the smaller and some in to the larger half. It seems to me that this can be done very properly; but that all may judge of this for themselves I give the process in detail. Thus in the vertical measurements of the head of the humerus there were 204 bones measuring 45 mm. and less, and 196 measuring more than 45 mm. The number measuring 45 mm. was nearly equal in both sexes, there being 16 male and 17 female. Thus if 4 of this group, 2 of each sex, were transferred from the smaller bones to the larger, there would be two divisions of 200 each, obtained by the transfer of only 1 per cent of the whole. In the smaller 200 there would be 23 (11.5 per cent) of the male and 177 (88.5 per cent) of the female. In the second half these figures would be reversed. In the series of the transverse diameter of the head of the humerus there were 191 bones measuring 41 mm. or less and 209 measuring more than 41 mm. Among the male bones were 14 of 42 mm., and among the female 18; by transferring 9 of these, 4 male and 5 female (2.25 per cent of the whole), to the smaller bones, we had 200 in each division arranged as follows: In the smaller 200 there were 22 male (11 per cent), and 178 female (89 per cent). In the larger 200 these figures were reversed. In the case of the head of the femur there were 195 of 46 mm. or less, and 205 of more than 46 mm. There were 37 measuring 47 mm., of which 22 were male and 15 female. By transferring 3 male and 2 female from the larger to the smaller bones, we once more have 200

in each division, the transfer being 1.25 per cent. In the smaller 200 there were 30 male (15 per cent), and 170 female (85 per cent). In the larger 200 these figures were reversed.

Surely these results are very convincing, and the manipulation by which two even halves are obtained quite justifiable.

Let us now inspect the curves. It stands to reason that there must be some overlapping. It is self-evident that the joints of all males cannot be larger than those of all females, even of the same race. I have already given the figures which show how surprisingly few of either sex pass beyond the average of the other sex; that is, how few male bones are below the female average and how few female bones above the male average. Now these curves show that if we suppress a small percentage composed of erratic individuals, the overlapping is remarkably small and restricted to a very narrow debatable ground. The curve of the vertical diameter of the humerus (Plate I) shows that the smallest male measurement is 41 mm. and the largest female 50 mm. Thus there is an overlapping extending through half the breadth of the two curves. There are 313 individual measurements overlapping (78.25 per cent). But the chart shows clearly that this wide spread of overlapping is due to a few aberrant specimens. If we take away only 9 male and 10 female (4.75 per cent), the number of overlapping bones is reduced to 64, or 16.80 per cent of the remaining 381. What is most remarkable is that after this elimination of extreme formations, the overlapping is limited to diameters of 45 and 46 mm.¹

The curve of the transverse diameter of the head of the humerus (Plate II) is very similar; 303 overlap (75.75 per cent), but if 7 male and 9 female (4 per cent) are thrown out only 68 bones (17.71 per cent) of the remainder overlap; and the overlapping is limited to bones measuring 41 and 42 mm.

The curve of the head of the femur (Plate III) is interesting inasmuch as there are fewer aberrant bones to remove and yet greater ultimate overlapping. Originally 313 bones (78.25 per cent), (precisely the same as in the vertical diameter of the humerus) overlap, but of these only 6 male and 3 female (2.25 per cent) are sufficiently isolated to justify their removal, after which 113 (28.90 per cent) of the remainder still overlap. Moreover, the overlapping includes three millimeters, namely, 46, 47 and 48 mm., instead of only two, as in both diameters of the humerus.

¹ That part of the curves represented by a continuous line shows them as they would be after this elimination.

Even the last is far from a bad result and shows that the size of the head of the femur has a great sexual significance, but distinctly less than that of the head of the humerus. The averages show the same thing, though less strikingly.

The main thesis seems thus to be established.

Let us now consider whether any particular shape of the articular head is more characteristic of either sex than another. As we have begun by assuming that the head of the femur is spherical, there can be no question about that; but as the head of the humerus has a long and a short diameter the question is possible. From some old observations on the glenoid cavity I had come to the conclusion that the head of the female bone is narrower than that of the male, and this is supported by the averages; but to such a minute degree as to be unworthy of consideration. As already stated, the female head of the humerus measures 87.51 per cent of the male in the vertical direction, and 87.28 in the transverse. The average difference in the former direction is 6.09 mm., and in the latter 5.68 mm. The transverse diameter is 91.59 per cent of the vertical in the male, and 91.35 per cent in the female. I then went to work on the individual differences between the vertical and the transverse diameters. The range of differences extends from —1 to 8 mm. The former means that in one single female bone the transverse diameter was 1 mm. greater than the vertical. No difference whatever was found in 3 males and 2 females. The difference was 1 mm. in 10 males and 13 females; the greatest difference, 8 mm., was found in 2 of each sex. Differences of 6 and 7 mm. were found much more frequently among the males than the females, as is to be expected from the greater size of the former. Running through a number of cases in both sexes in which the difference was slight and of others in which it was large, I was quite unable to see anything that pointed to a sexual difference in this respect sufficiently marked to be worth recording. I was unequally unsuccessful in trying to ascertain whether, regardless of sex, a long or a round head of the humerus was to be expected more frequently in large or small bones.

I endeavored to ascertain whether the difference between the head of the humerus and that of the femur was greater in one sex (probably the female) than the other, but I failed again. The average difference of the vertical diameters showed a difference to the advantage of the femur of .92 mm. for the male, and 1.17 mm. for the female. On the other hand, the transverse diameter gave the femur an advantage of 5.02 mm. in the male, and 4.86 in the female. Study of indi-

vidual cases of large and small bones did not give any encouragement to undertake the labor of elaborate tabulation.

We come now to the very important feature of this series of observations that the measurements were made on bones with the articular cartilage not only in place, but not dried. As above mentioned, this is the proper method, as it shows the parts as they are in life, giving the true size of the joints; but there is the serious consideration that most observations are made on dried bones, so that one needs to know what allowance is to be made for the absence of the cartilage. What complicates the matter is that the conditions for the humerus and for the femur are not the same. In measuring the practically globular head of the femur, the greatest diameter passes through the centre of the sphere and traverses the whole thickness of the cartilage on both sides of the head. With the humerus the conditions are very different. Both the long and the short diameter, especially the former, run through the bone just at the insertion of the shaft into the head, that is, at the border of the articular cartilage which narrows around the margin of the head so as to be extremely thin. I have been at great trouble to find a method of determining how much to allow for the cartilage and can find none that is satisfactory. I think that from 2 mm. to 3 mm. should be allowed for the femur, and that .5 or 1 mm. is enough for the humerus.

When the work was far advanced I regretted that I had contented myself with measuring only the heads instead of taking the length and perhaps the thickness of the bones. Although as a practical anatomist I know that no one would think of determining the sex of either of these bones by its length, I felt that it would be difficult to answer anyone who might ask how I could be certain that there is a greater discrepancy between the articular heads of the bones than between their lengths. I had recourse to Dr. Hrdlicka of the National Museum of Washington, who came to my rescue with measurements of bones of 200 white adults, 100 of each sex, made by him at the Medical Department of Columbia University of New York. The sources from which these bones came are perhaps a little more diverse than those that lead to Boston, but not, I believe, very much so. I have already shown that the dissecting room material in Boston does not in the least represent any single race.* That from New York is only somewhat more heterogenous. In short this collection of measurements of the length is, failing that of the bones on which the joints were measured, as good as could be expected. It is quite good enough for the very general conclusions I shall draw. I wish to express my deep obligations to Dr. Hrdlicka for his kind generosity in this matter, by which I am enabled to compare with this series of meas-

urements of 400 articular heads of humerus and as many of that of the femur a series of the length of 200 humeri and 200 femora, equally divided between the sexes. The length of the thigh bone was taken by the bicondylar method. The greatest possible length of the arm bones was recorded. All the measurements were of bones of the right side. It is very evident that the difference between the bones of the arm and thigh in the matter of length are much less important sexually than those of the diameters of the heads. In comparing the curves (Plates IV and V) it is to be remembered that the observation of the lengths are but half as numerous as those of the joints.

The average length of the male humerus is 32.46 cm., that of the female 29.98 cm., which is 92.36 per cent of that of the male. The average male femur measures 44.95 cm., the female 41.55 cm., or 92.44 per cent of the former. The diameter of the head of the female femur approaches that of the male rather more closely than either of the diameters of the head of the humerus, yet its percentage is only 88.24. Before plotting the curves, the millimeters were suppressed, each bone being recorded as at the nearest centimeter. Cases which came at precisely half a centimeter were put at the lower mark.

I pointed out in the case of the joints how very few male ones were below the female average, and how very few female ones above the male average. It is easy to see by consulting the curves of the lengths, in which the averages have been marked, that though there are only half as many observations there are, especially in the case of the female femora, decidedly more beyond the line. The contrasts between the two sets of series when divided into a smaller and a larger half are very instructive. Let us take first the length of the humerus. If the dividing line be put between 31 and 32 cm., we find 119 in the first division and 81 in the second. In the former there are 22 male and 23 female bones measuring 31 cm. If we transfer 9 males and 10 females (9.5 per cent of the whole) we have 100 in each division. There are 24 males in the shorter 100 and 24 females in the longer 100, *i. e.*, 24 per cent of each sex in the wrong half. If we draw the dividing line for the femora between 43 and 44 cm., we have 118 in the shorter division and 82 in the longer. There are 16 male and 16 female bones measuring 43 cm. If we transfer 9 of the former and 9 of the latter (9 per cent) to the longer division we again have two divisions of 100. Among the shorter 100 are 27 (27 per cent) male, and among the longer 27 female (27 per cent). A glance at the former statement of this manipulation with the diameters of the joints will show that the percentage of specimens transferred was insignificant, never over 2.25 per cent, and the result much better.

Let us now compare the curves. At first sight they give no hint of the difference which analysis reveals. The curves of the joints show an original overlapping in the order in which they are given of 78.25, 75.75 and 78.25 per cent respectively. The curve of the length of the humerus shows 89 per cent overlapping, and that of the femur 82 per cent, but in the case of the joints the amount of overlapping was wonderfully reduced by the elimination of a few stragglers, respectively 4.75, 4. and 2.25 per cent, the percentages of overlapping dropping to 16.80, 17.71 and 28.90 of the remainder. In the tables of the length a much larger elimination brings about much less satisfactory results. Thus with the humerus the elimination of 15 bones (7.5 per cent), reduced the overlapping of the remainder only to 41.62 per cent; and with the femur the rejection of 15 bones (7.5 per cent) still leaves the overlapping of the remainder at 46.49 per cent. This is a very significant difference.

Thus it is demonstrated that the difference in the size of the articular surfaces in the sexes is very much more marked than that of the length of the respective bones. Although I have not established it by figures, I have no hesitation in saying that it is also much more marked than the difference in the thickness of bones. A striking illustration of this is furnished by the photograph (Plate VI) of a male and a female humerus, side by side, so placed as to show the articular heads as well as possible. There is very little difference in the length and in the thickness, but the much greater size of the joint shows at a glance which is the male. What is most interesting is that the male bone came from the body of a very puny young man of nineteen, who being blind, had passed his life in an almshouse doing very little work. I remember him particularly from the fact that he had but one kidney. My personal recollection of the body of the woman from which the female bone was taken is less sharp; but she is said to have been of uncommonly powerful make. The muscular ridges on the bone confirm this, yet a glance at the head is so conclusive that it is needless to mark which is which.

I have devoted a good deal of time to the glenoid cavity of the scapula, and more or less to other joints in the extremities. Although I cannot speak by the book, I feel very sure that the law which is deduced from the humerus and femur will be found to apply, though perhaps with more exceptions, to all the joints of the extremities. It should not need to be said that it is the province of such a law to be a guide to the expert who will apply with discretion. Absolute certainty as to the sex of bones does not exist in all cases. The judgment matured by long observation is certainly better than any rigid adherence to a mathematical law. None the less, in many cases, the law of the relative smallness of the female

joints will go far to show the probability of what one may hesitate to affirm as absolutely certain.

The following conclusions seem justified by this study of 400 humeri and femora of white adults:

The heads of the humerus and femur are relatively small in woman. Probably the same may be said of other joints.

Dorsey's investigations show that this anthropological law applies also to savage races (or at least to some of them).

The number of measurements of male joints smaller than the average female joint and of female ones larger than the average male is insignificant. In the transverse diameter of the head of the humerus the combined number is only 1.25 per cent, and in the head of the femur only .05 per cent.

By rejecting a few aberrant specimens the overlapping in the curves of both diameters of the humerus is reduced to about 17 per cent, and is limited to joints measuring 45 and 46 mm. vertically and to those measuring 41 and 42 mm. transversely.

The head of the femur is somewhat less characteristic, but still very valuable as a guide to the sex.

(These measurements were made with the articular cartilage in place and still fresh.)

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DESCRIPTION OF PLATES I TO VI.

PLATE I.—The binomial curves of the vertical diameter of the heads of 200 male and 200 female humeri expressed in millimeters. The female curve is on the left.

PLATE II.—Ditto of the transverse diameter of the same.

PLATE III.—Ditto of the greatest diameter of the head of the femur.

PLATE IV.—The binomial curves of the length of 100 male and 100 female humeri (Dr. Hrdlicka's) expressed in centimeters. The female curve is on the left.

PLATE V.—Ditto of the length of the femur (Bicondylar) (Dr. Hrdlicka's).

The part of the curves drawn with a continuous line represents what they would be after the elimination of certain aberrant overlapping bones.

PLATE VI.—The humerus of a strong woman and of a puny man. The sex is evident from the size of the heads of the bones.

APPENDIX.

It is hoped that these figures may be of use. The humerus and femur in the same line always came from the same body, but no regard has been paid to the side. The measurements include the articular cartilage which was in good condition.

DIAMETERS OF HEAD OF HUMERUS AND FEMUR.

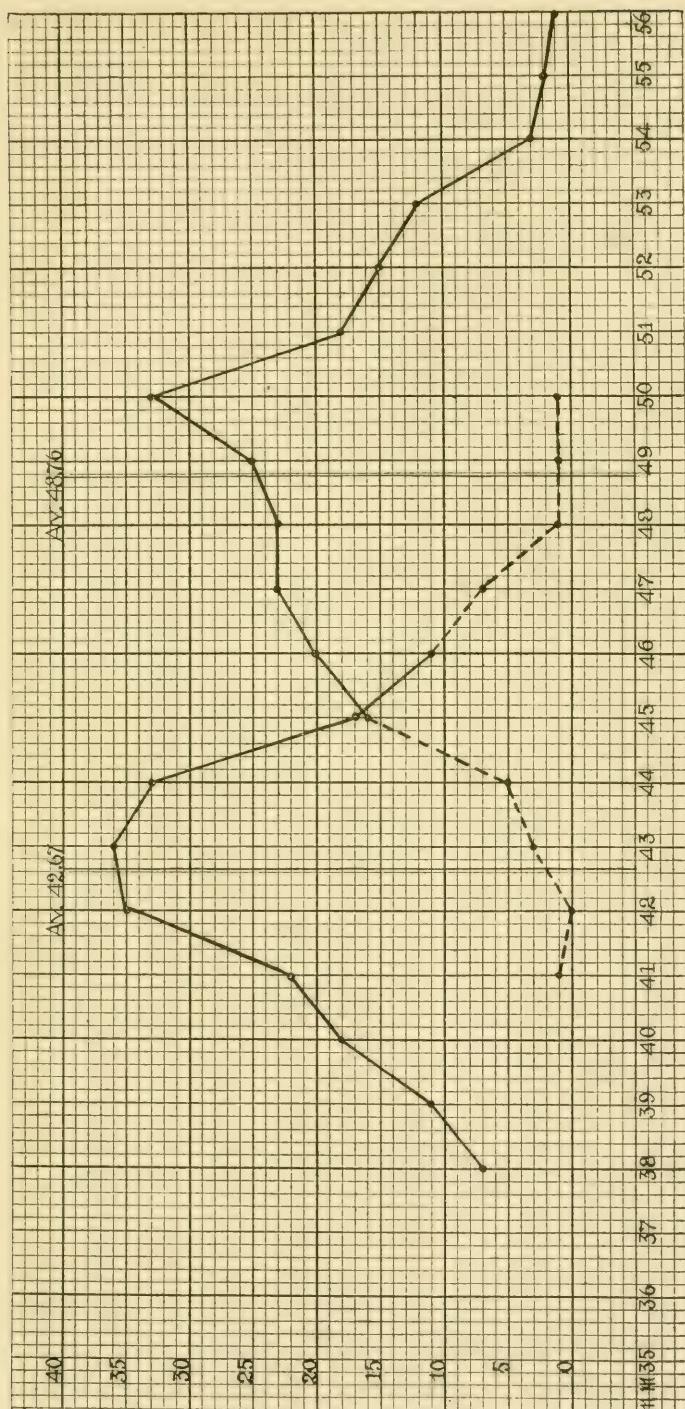
MALE.

HUMERUS.		FEMUR.		HUMERUS.		FEMUR.		HUMERUS.		FEMUR.	
Vert.	Trans.	Vert.	Trans.	Vert.	Trans.	Vert.	Trans.	Vert.	Trans.	Vert.	Trans.
1	53	47	49	68	46	45	50	135	46	41	51
2	52	48	48	69	46	40	46	136	49	46	51
3	43	39	46	70	50	44	50	137	49	44	46
4	45	42	47	71	48	43	48	138	49	49	56
5	45	44	47	72	54	52	52	139	52	50	51
6	46	43	46	73	52	47	52	140	47	44	46
7	52	47	54	74	51	45	54	141	52	46	50
S	45	40	46	75	55	48	53	142	50	46	51
9	50	46	49	76	47	44	47	143	49	43	49
10	46	43	48	77	46	43	49	144	48	47	51
11	48	46	48	78	45	43	46	145	49	43	49
12	47	45	48	79	53	48	54	146	50	43	50
13	50	45	49	80	45	45	49	147	51	47	51
14	49	44	50	81	46	44	54	148	51	46	52
15	47	44	51	82	49	45	54	149	47	45	48
16	45	42	48	83	47	45	46	150	52	49	52
17	50	43	46	84	46	42	46	151	48	42	48
18	44	43	49	85	50	44	52	152	53	46	53
19	41	38	42	86	45	41	46	153	50	45	55
20	48	44	47	87	51	46	52	154	51	45	52
21	48	44	52	88	46	43	46	155	49	44	53
22	45	42	45	89	51	47	48	156	51	47	52
23	48	46	49	90	51	46	53	157	47	43	50
24	46	42	47	91	52	46	52	158	47	44	50
25	43	42	44	92	46	43	50	159	53	48	51
26	46	43	48	93	53	44	52	160	49	44	50
27	45	43	56	94	45	43	47	161	45	41	49
28	48	43	47	95	50	43	46	162	46	43	46
29	43	41	47	96	48	44	50	163	55	47	54
30	52	46	54	97	46	42	46	164	46	41	49
31	50	46	51	98	46	40	51	165	44	42	46
32	44	42	46	99	54	49	53	166	48	46	51
33	51	44	50	100	47	44	47	167	53	51	55
34	46	41	48	101	45	44	47	168	50	47	52
35	47	43	50	102	53	46	51	169	47	43	48
36	52	47	51	103	46	41	46	170	51	48	50
37	44	41	45	104	52	47	52	171	47	44	49
38	52	44	49	105	50	43	51	172	50	45	50
39	49	42	46	106	45	44	48	173	50	47	51
40	47	43	47	107	52	50	51	174	49	43	49
41	54	49	54	108	48	45	47	175	47	45	48
42	49	45	49	109	48	45	48	176	47	43	48
43	52	48	50	110	51	48	54	177	50	45	53
44	50	45	51	111	52	49	51	178	47	45	50
45	50	50	51	112	48	43	47	179	47	43	48
46	51	46	51	113	50	43	50	180	53	47	53
47	45	41	47	114	50	45	49	181	50	44	51
48	51	48	52	115	49	45	47	182	52	46	51
49	48	45	52	116	45	38	49	183	49	43	51
50	48	42	47	117	48	45	50	184	50	46	53
51	49	48	52	118	50	46	51	185	47	41	48
52	49	46	47	119	49	45	49	186	50	43	51
53	47	45	47	120	48	45	50	187	49	45	50
54	50	44	49	121	49	44	50	188	44	42	46
55	50	46	49	122	50	49	51	189	46	43	46
56	48	43	51	123	51	49	50	190	47	44	48
57	49	44	53	124	47	45	47	191	53	46	52
58	50	45	49	125	49	45	49	192	49	46	56
59	48	42	47	126	53	49	53	193	51	45	50
60	51	48	51	127	51	44	52	194	49	46	51
61	53	47	53	128	56	50	56	195	53	47	51
62	49	46	53	129	51	47	47	196	49	45	49
63	48	44	48	130	50	47	52	197	48	47	52
64	45	40	44	131	46	41	44	198	50	45	49
65	48	45	51	132	50	46	52	199	50	45	53
66	48	43	53	133	50	45	50	200	50	45	50
67	47	44	48	134	47	43	47				

DIAMETERS OF HEAD OF HUMERUS AND FEMUR.

FEMALE.

HUMERUS.		FEMUR.		HUMERUS.		FEMUR.		HUMERUS.		FEMUR.	
Vert.	Trans.	Vert.	Trans.	Vert.	Trans.	Vert.	Trans.	Vert.	Trans.	Vert.	Trans.
1	42	39	42	68	45	40	48	135	43	39	46
2	43	39	42	69	43	40	42	136	44	39	46
3	42	37	44	70	38	36	39	137	47	43	48
4	43	38	40	71	40	36	40	138	44	39	45
5	42	39	42	72	40	38	42	139	47	43	47
6	46	42	46	73	44	40	46	140	40	38	44
7	43	38	42	74	39	36	40	141	44	39	47
8	44	42	46	75	43	38	45	142	42	40	46
9	44	40	49	76	45	42	48	143	44	41	45
10	41	38	41	77	38	35	39	144	43	40	43
11	45	40	44	78	42	37	40	145	45	37	44
12	46	45	47	79	45	39	45	146	41	39	45
13	40	37	40	80	42	38	42	147	44	38	45
14	47	41	47	81	44	39	47	148	40	36	44
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16	41	37	44	83	42	38	39	150	41	37	44
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18	38	37	39	85	43	42	46	152	42	36	44
19	43	41	43	86	44	36	41	153	41	37	43
20	43	41	42	87	41	37	46	154	44	39	46
21	42	37	41	88	44	43	44	155	39	35	42
22	41	39	41	89	40	37	45	156	44	41	48
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64	47	41	48	131	44	41	47	198	43	39	45
65	42	38	47	132	41	37	42	199	45	41	46
66	44	40	44	133	40	38	44	200	42	41	45
67	39	35	38	134	46	40	46				



MALE

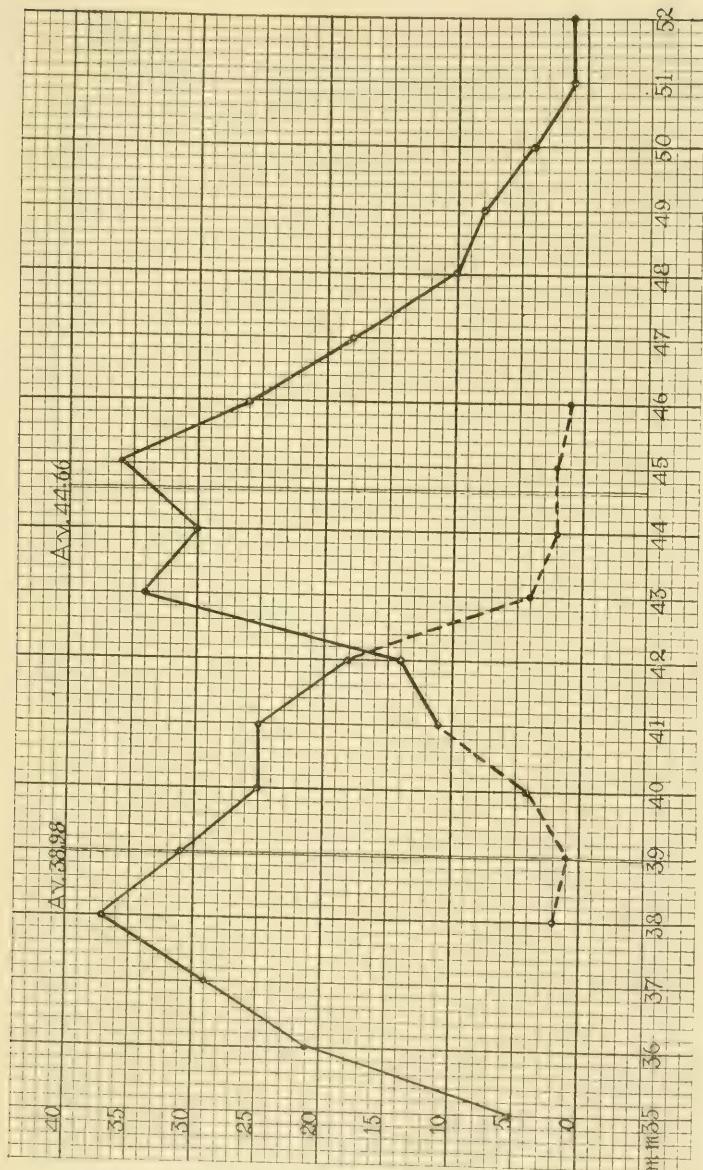
FEMALE

VERTICAL DIAMETER OF THE HEAD OF THE HUMERUS

SIZE OF ARTICULAR SURFACES OF LONG BONES

T. DWIGHT

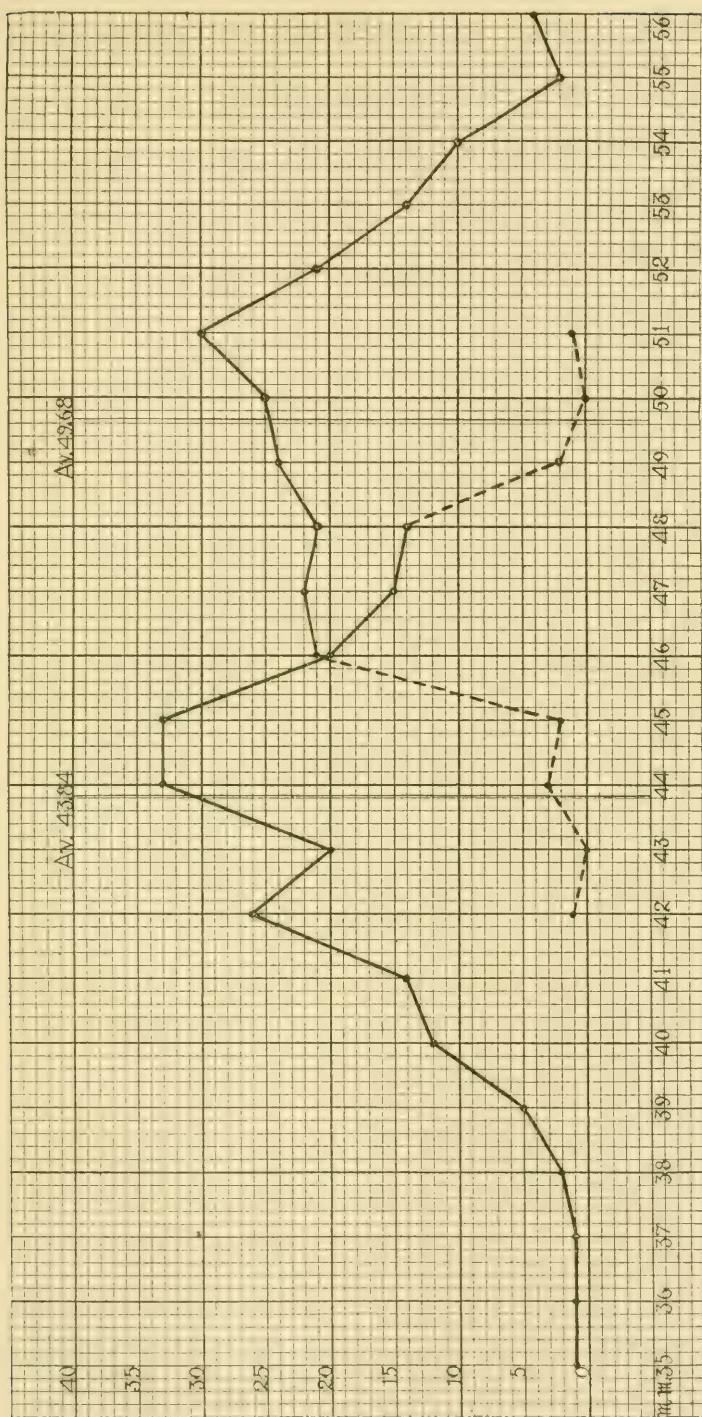
PLATE II



MALE

TRANSVERSE DIAMETER OF THE HEAD OF THE HUMERUS

FEMALE



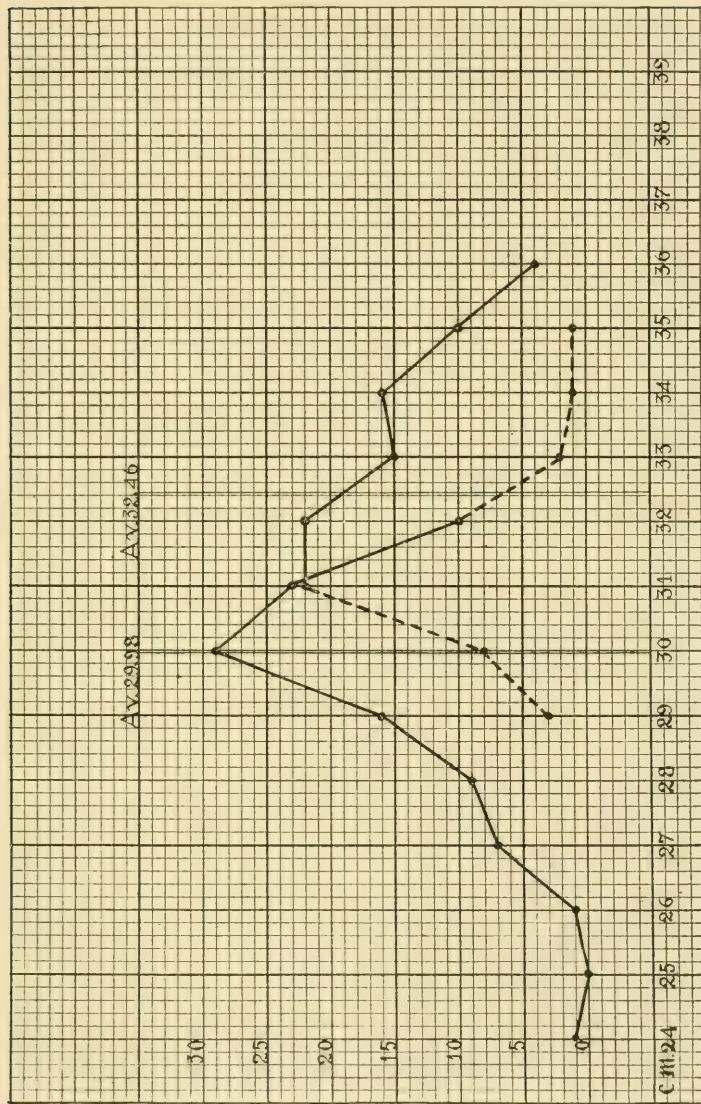
MALE

DIAMETER OF THE HEAD OF THE FEMUR

FEMALE

SIZE OF ARTICULAR SURFACES OF LONG BONES
T. DWIGHT

PLATE IV



MALE

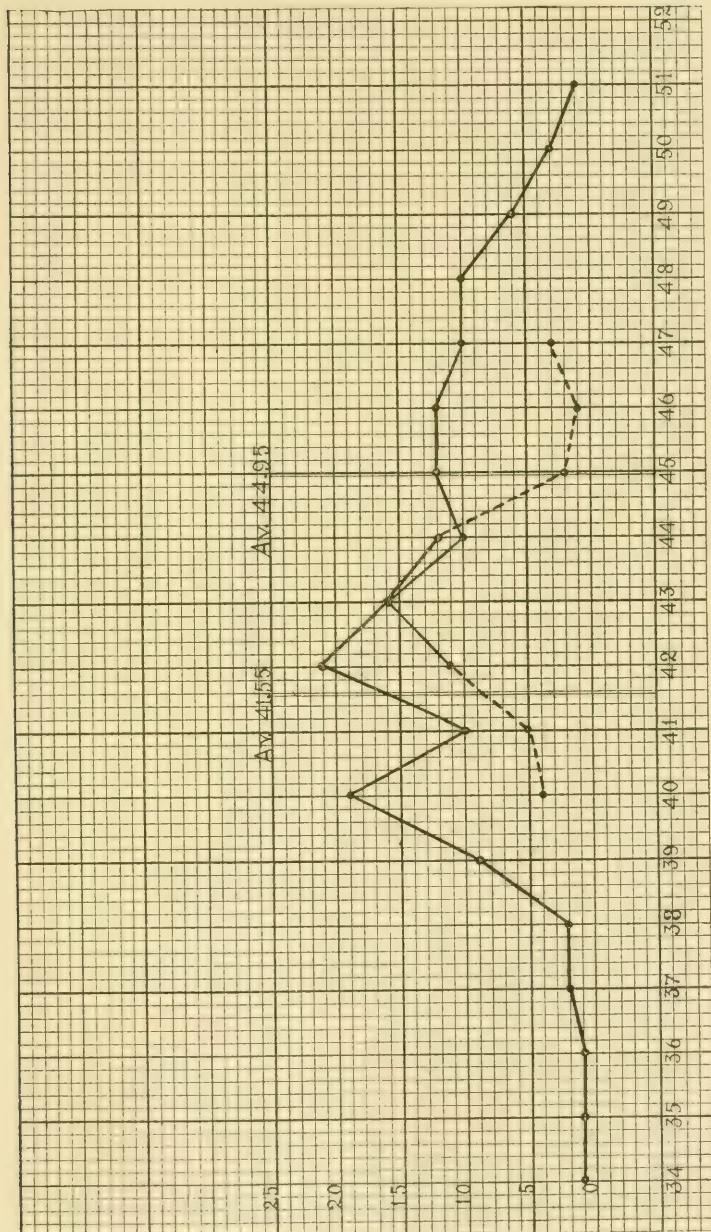
LENGTH OF THE HUMERUS

FEMALE

SIZE OF ARTICULAR SURFACES OF LONG BONES

PLATE V

T. DWIGHT



MALE

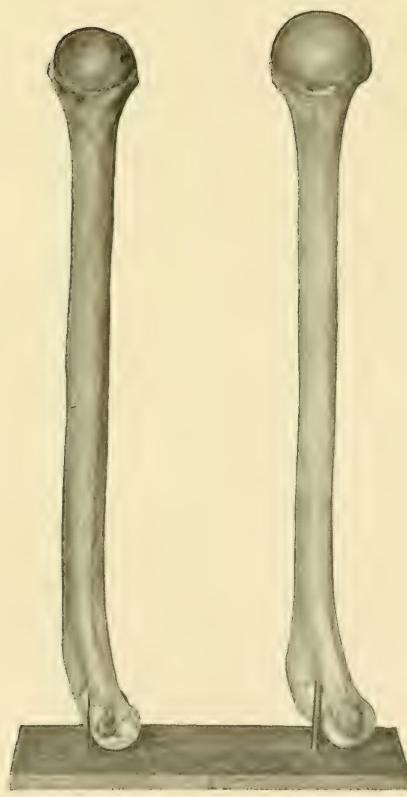
LENGTH OF THE FEMUR

FEMALE

SIZE OF ARTICULAR SURFACES OF LONG BONES

PLATE VI

T. DWIGHT



THE PHYLOGENY OF THE CRURAL FLEXORS.

BY

J. PLAYFAIR McMURRICH.

From the Anatomical Laboratory of the University of Michigan.

WITH 14 TEXT FIGURES.

In an earlier paper (1903) I presented the results of a comparative study of the flexor muscles of the antibrachial region and showed that it was possible to trace step by step the changes by which the arrangement occurring in the Urodeles amphibia was converted into that characteristic of the mammalia. In the amphibia the muscles in question possess a definite arrangement in layers and it was shown that these layers have a fundamental significance, since, notwithstanding the almost innumerable modifications and differentiations which they present in higher forms and the apparently enormous differences which exist between the amphibian and mammalian forearm musculatures, yet the layers could be recognized throughout and consequently afforded a basis for the reconstruction of the phylogeny of the mammalian muscles.

It became of interest, consequently, to ascertain whether a comparative study of the crural flexors would reveal a similar fundamental arrangement in layers and so afford a basis for their phylogenetic reconstruction, and, if so, an opportunity for a satisfactory consideration of the much-discussed question of the serial homology of the arm and leg musculature. In the following pages the results of such a study are recorded in so far as they bear upon the first of the two problems mentioned, namely, the phylogeny of the crural flexors. The question of the serial homology of the arm and leg musculature I hope to discuss later in connection with some other general questions relating to the morphology of the vertebrate limb.

The methods and forms employed in the present study were essentially the same as those made use of in the investigation of the arm muscles. The arrangement and relations of the muscles were studied in serial transverse sections and the forms employed were *Amblystoma tigrinum* as a representative of the Urodele amphibia, *Scincus sp?* as

a representative of the reptilia and the opossum (*Didelphys virginiana*),¹ the mouse, the cat and man as representatives of the mammalia.

I. THE CRURAL FLEXORS OF THE URODELOUS AMPHIBIA.

A transverse section through the upper part of the crus of *Ambystoma tigrinum* shows the arrangement of parts represented in (Fig. 1). Superficially upon the posterior surface of the section is seen a strong, somewhat crescentic, muscular mass, which, employing a terminology consistent with that used in the description of the antibrachial muscles,

may be termed the *plantaris superficialis medialis* (*Psm*). It may be remarked in passing that while the terms used by Eisler, 95, in his careful and suggestive paper on the homology of the extremities are also employed here, their application is very different, since Eisler has failed to recognize the muscle now referred to as a distinct constituent of the crural flexor mass, that muscle which he terms the *plantaris superficialis* lying beneath the muscle now under consideration and forming what I shall describe as the *plantaris profundus III*.

In addition to this most superficial muscle, there is upon the outer side of the leg another muscle (*Psl*) which must also be referred to the superficial layer and which may be

FIG. 1.—Transverse section through the upper part of the crus of *Ambystoma tigrinum*. *F*, fibula; *f*, ramus superficialis fibularis; *FT*, fibulo-tarsalis; *I*, interosseus; *m*, ramus superficialis medialis; *p*, ramus profundus; *Pp I-III*, plantares profundi I-III; *Pp III m*, plantaris profundus III minor; *Psl*, *Plantaris superficialis lateralis*; *Psm*, *plantaris superficialis medialis*; *T*, tibia.

termed the *plantaris superficialis lateralis*.

Beneath the superficial layer formed by these two muscles lies a larger

¹ As on a former occasion, I am indebted to my friend, Dr. C. F. W. McClure, for the opossum material which was used, and I desire to express my appreciation of his courtesy in placing at my disposal material without which my studies would necessarily have been very incomplete.

To my assistant, Mr. F. S. Bachelder, I am greatly indebted for assistance in the work, since he kindly undertook the preparation of all the serial sections that were required. He also studied with me the arrangement of the muscles and nerves of the amphibian crus and so much of this paper as treats of these structures is to be regarded as our joint work.

muscle mass whose fibres have a markedly oblique direction and which will be spoken of as the *plantaris profundus III* (*Pp^{III}*). Beneath the fibular border of this there is a small oval muscle whose fibers are cut transversely and which is the *fibulo-tarsalis* (*FT*), while opposite its tibial border there is a very slender muscle which may be termed the *plantaris profundus III minor* (*Pp^{III}m*). Still more deeply seated are two layers of muscle whose fibers are directed obliquely downward and tibia-wards, the *plantares profundi II* and *I* (*Pp^{II}* and *Pp^I*), and finally, extending almost directly across between the fibula and tibia is a muscular sheet which may be termed the *m. interosseus* (*I*).

A comparison of such a section with one taken through the forearm of *Amblystoma* will show that a remarkable similarity exists between the two. There is exactly the same number of layers and the same direction of the fibers in the different layers. Indeed, the resemblance is so close that the two sections might easily be confused in a casual examination. A discussion of their resemblances and the significance of these will, however, be postponed until a later occasion, and I shall pass on now to a consideration of the various muscles mentioned above.

And first as to the *plantaris superficialis medialis* (*Psm*). As already noted, this muscle is not regarded by Eisler as a part of the crural musculature and in this view he is in agreement with his predecessors. The muscle is continuous above with the lower end of the muscle named *ischio-flexorius* by Hoffmann, 73, the caudo-pedal by Humphry, 72, and the external flexor of the crus by Perrin, 93, and, indeed, is the terminal part of that muscle. From dissections there is little reason to regard it otherwise than as these authors have done, but its relations as seen in sections, especially when these are compared with sections through the forearm, speak so strongly for the view here set forth that I have no hesitation in advancing it. And especially so since there are two other facts bearing favorably upon it, namely (1) the insertion of the muscle into the plantar aponeurosis, which occurs a short distance below the knee joint, and (2) the fact that in *Amblystoma* the *ischio-flexorius* is crossed at the knee joint by a well-marked tendinous inscription, which marks, I believe, the line of junction of the *ischio-flexorius* proper with the *plantaris superficialis medialis*. The *ischio-flexorius* of Hoffmann is, according to this view, a compound muscle formed by the end to end union of a true *ischio-flexorius* with a *plantaris superficialis medialis*.²

² If I understand aright Humphry's, 72, description of the caudo-pedal muscle of *Cryptobranchus*, there is in this form also a tendinous inscription in the muscle in the neighborhood of the knee joint. If this be so, it is

The *plantaris superficialis lateralis* (*Psl*) is a muscle which has invariably been described as the femoral head of the large superficial muscle which I term the *plantaris profundus III*. From the remarkable similarity of the muscles of the forearm and crus in the urodele amphibia it might be expected that the same constancy in the relations of the superficial and deep flexors of the leg to the femur and crural bones as obtained in the corresponding muscles of the crus in their relations to the humerus and antibrachial bones would be found, and it was on this ground that I was first led to refer this muscle to the superficial layer and to regard it as distinct from the *plantaris profundus III*. Further study only served to confirm the correctness of this view by revealing a consistent phylogeny for the crural flexors based upon it.

The muscle takes its origin from the flexor surface of the external condyle of the femur and is separated by a distinct interval from the upper part of the origin of the *plantaris profundus III*. It passes down the fibular side of the leg, quite distinct from the *plantaris profundus* and passes over into a rather feeble tendon, which is inserted into the outer border of the fibula near its lower extremity. Throughout its whole extent, therefore, it is distinct from the *plantaris profundus III* in *Amblystoma*.

The *plantaris profundus III* (*Pp^{III}*) is the largest of all the muscles of the crus and is described by Humphry, 72, as the *flexor sublimis digitorum*, by Hoffmann, 73, as the *femoro-fibulæ-digi* I-V, by Perrin, 93, as the *external flexor of the digits* and by Eisler, 95, as the *plantaris superficialis major*, all these authors, as has already been noted, including in the muscle the *plantaris superficialis lateralis*. It arises in *Amblystoma* from the posterior surface of the upper part of the fibula and its fibers are directed downwards and somewhat tibially to be inserted into the under (dorsal) surface of the plantar aponeurosis, through which it acts upon the digits.

From the upper part of the tibial border of this muscle a slender slip (*Pp^{III}m*) separates and passes almost vertically downwards to fade out in connective tissue in the neighborhood of the ankle joint, in close proximity to the tibial border of the *plantaris profundus I*. This is evidently the muscle described by Eisler as the *plantaris superficialis minor* and has apparently been overlooked by Perrin. It seems to be,

interesting to note that in the caudo-pedal of *Cryptobranchus* there is not only an end to end union of the *ischio-flexorius* proper and the *plantaris superficialis medialis*, but also of the former and what may be termed an *ischio-caudalis*.

in *Amblystoma*, a derivative of the *plantaris profundus III* and may be termed the *plantaris profundus III minor*.

The fibulo-tarsalis (*FT*) arises from the posterior surface of the upper part of the fibula and extends vertically down the crus, lying immediately behind the fibula, to be inserted into a strong tendinous band which extends transversely across the sole of the foot at about the level of the distal row of tarsal bones. This muscle is the fibulo-*plantaris* of Eisler, the deep common flexor of the phalanges of Perrin, the *fibulæ-metatarsi et digiti I-V* of Hoffmann and the *flexor profundus digitorum* of Humphry.

The *plantaris profundus II* (*Pp^{II}*) also arises from the posterior surface of the fibula and takes an oblique direction downwards and tibially to be inserted into the deeper (dorsal) surface of the plantar aponeurosis. It is the *plantaris profundus I* of Eisler, the internal flexor of the digits of Perrin and the *femoro-fibulæ metatarsi I-III* of Hoffmann.

The *plantaris profundus I* (*Pp^I*) arises from almost the whole length of the fibula and from the tarsus and is directed downward and tibially to be inserted into the lower end of the tibia, into the *tibiale* and the *tarsale I*; I did not find any insertion into the plantar aponeurosis in *Amblystoma*. This is the muscle described by Perrin as the direct rotator of the foot, and is apparently represented in *Menopoma*, according to Eisler, by four more or less distinct muscles which have been named the *plantares profundi II* and *III*, the *fibulo-tibialis* and the *fibulo-tarsalis*. Humphry and Hoffmann have not recognized it as distinct from the *plantaris profundus II*.

Finally, the *interosseus* (*I*) is a strong band of muscle fibers which extend almost directly across between the tibia and fibula, occupying the position of the interosseous membrane of the higher mammalia. It is the *pronator tibiæ* of Humphry and the *fibulæ-tibialis* of Hoffmann.

In the study of the arm flexors much light was thrown upon their phylogenetic modifications by their nerve supply and the same holds good for the crural flexors. It must be remembered, however, that with the modifications which the muscles undergo in the various groups, a modification of the nerve trunks also occurs, and in making use of the nerve supply for the identification of muscle equivalents in the different groups, these changes in the paths followed by the nerve fibers must be taken into consideration. The final test in the identification of a motor nerve is its end organ, the muscle; that is a definite quantity in the problem. But the path by which a given nerve reaches its end organ is not necessarily the same in all cases; the nerve, as a rule, will seek the most direct route

to its destination, but that route need not be exactly the same in all forms. The tendency, however, is largely towards conservatism, and even when the bulk of the fibers composing a given nerve trunk, adopt, in the higher vertebrates, a new path, some will be apt to retain the original course and so serve as guides for the determination of topographic relationships. I have elsewhere (1903, pp. 466-7) expressed in general terms the conclusions in regard to the value of nerve supply in determining muscle homologies to which my studies of the muscle and nerves of the forearm have led me.

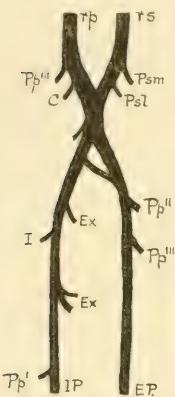


FIG. 2.—Diagram of the crural nerves in *Ambystoma tigrinum*. *C*, cutaneous branch; *EP*, external plantar; *Ex*, branches to extensor surface; *I*, branch to interosseous; *IP*, internal plantar; *PpI-III*, branches to plantares profundi I-III; *Psm*, branch to plantaris superficialis medialis; *rp*, ramus profundus; *rs*, ramus superficialis.

In *Ambystoma*, immediately above the knee joint, two distinct nerve trunks occur upon the posterior surface of the leg (Fig. 2). They are formed by the division of the sciatic nerve after it has given off the peroneal nerve and are what Humphry, 72, has termed the internal and external popliteal nerves. They do not, however, correspond in composition to the nerves so named in the mammalia, and for this reason they will be spoken of here as the *ramus plantaris profundus* (*rp*) and the *ramus plantaris superficialis* (*rs*). They lie, at first, one on either side of the sciatic artery, but as they are traced downwards the ramus profundus passes slightly laterally so as to come to lie in front of the ramus superficialis, and a little later the two stems fuse, only to separate again, some interchange of fibers apparently taking place, however, during the fusion, and a further interchange is carried out by means of a cross connection between the two stems a little lower down.

From the ramus superficialis above the fusion branches are given off to the plantaris superficialis medialis (*Psm*) and to the plantaris superficialis lateralis (*Psl*), and below the fusion to the plantaris profundus III (*Pp^{III}*) and the plantaris profundus II (*Pp^{II}*). Just as these nerves are given off the stem is passing over the upper border of the plantaris profundus III and its course is then downwards and outwards between the fibulo-tarsalis and the plantaris profundus II (Fig. 1, *f*), or to a certain extent through the substance of the fibulo-tarsalis. It thus reaches the fibular side of the crus and descends towards the foot, lying between the lateral border of the fibulo-tarsalis and the origin of the plantaris profundus II. Beyond this it will be unnecessary to follow it at present.

The ramus profundus (*rp*) gives off above the fusion a branch to

the plantaris profundus III (*Pp^{III}*) and below the fusion a branch (*E.x.*) which passes downward and forward through a notch on the crest of the tibia and is supplied to the muscle which has been termed the tibialis anticus. The main stem then gives off a branch (*I*) to the interosseus, and having in its downward course passed successively over the upper border of the plantares profundi III-I, it passes over the upper border of the interosseus and is continued downward on the extensor surface of that muscle (Fig. 1, *p*). Before reaching the foot it gives off a branch and then divides into two stems, one of which, together with the branch, passes to the muscles upon the dorsum of the foot, while the other passes backwards beneath the lower border of the interosseous muscle, gives off a branch to the plantaris profundus I and continues onward to be distributed to the plantar surface of the foot.

In order to understand the significance of this arrangement of the nerves it will be necessary to compare it with what occurs in the arm. In this but a single nerve trunk, the brachialis longus inferior, enters the flexor surface of the antibrachium and it divides into a ramus profundus and a ramus superficialis. The former has a course almost identical with that of the ramus profundus of the crus and supplies the pronator quadratus and the palmaris profundus I, which have the same topographical relations as the interosseus and plantaris profundus I supplied by the ramus plantaris profundus. The latter nerve, however, also contains some extensor fibers which are lacking in the deep nerve of the antibrachium, the separation of the præaxial and postaxial fibers having taken place higher in the arm than in the leg.

The ramus superficialis of the antibrachium divides into two portions, a ramus superficialis medialis and a ramus superficialis ulnaris, the latter of which possesses relations similar to those of the ramus plantaris superficialis after it has given off its branches to the plantares profundi III and II. It would seem, therefore, that these branches may well be regarded as equivalent, in part at all events, with the ramus superficialis medialis of the arm, while the main stem below their origin may be considered the equivalent of the ramus superficialis ulnaris and be termed the *ramus superficialis fibularis*.

But the ramus superficialis medialis of the arm supplies not only the palmares profundi III and II, but also the palmaris superficialis. In the leg the branches which are distributed to the muscles which I have identified as forming the plantaris superficialis, are given off from the ramus superficialis above the point of its fusion with the ramus profundus, so that a difference from the arrangement in the arm exists in that there is no concrete ramus superficialis medialis, its branches arise

ing independently at different levels. And, furthermore, a more important difference exists in the origin of a branch to the plantaris profundus III from the ramus profundus above its point of fusion with the ramus superficialis. This might seem to vitiate any direct homology between the ramus plantaris profundus and the ramus palmaris profundus, but, on the other hand, it may be a part of the same lack of differentiation of the plantar nerves which is evidenced in the retention of extensor fibers in the ramus plantaris profundus. In *Cryptobranchus*, according to Humphry, 72, both the ramus profundus and the ramus superficialis send branches to the plantaris profundus III, and in *Menopoma*, to judge from Eisler's figures, 95, the two stems separate only at the upper border of the plantaris profundus II, from which it may be presumed that the branches to the plantaris profundus III are given off from the common stem above the bifurcation. Whether the high or the low bifurcation be the more primitive condition, it is difficult to say, but it is at least plausible to suppose that the fusion of the two trunks in *Ambystoma* presents opportunities for the transference of fibers destined for the plantaris profundus III (and possibly II) from the ramus profundus to the ramus superficialis, since, apparently, the fibers which form the lower cross connection between the two stems are destined for the supply of the plantaris profundus II.

However that may be, it seems clear that in the plantar nerves there is less definiteness in the differentiation of the nerve fibers into special trunks than occurs in the palmar nerves, a fact which is shown by the inclusion of praaxial fibers in the same trunk with postaxial ones throughout the entire length of the crus and by the inclusion of fibers destined for superficial muscles in the same trunk with others for the deep muscles.

Tabulating the nerve supply of the plantar muscles according to the origin of the fibers from the two main stems the following arrangement is obtained :—

Plantaris superficialis medialis,	}	Ramus superficialis.
Plantaris superficialis lateralis,		
Plantaris profundus III (in part),		
Fibulo-tarsalis,		
Plantaris profundus II,	}	Ramus profundus.
Plantaris profundus III (in part),		
Plantaris profundus I,		
Interosseus,		

but, if the interpretation of the plantar nerves given above on the basis of a comparison with the arm nerve be accepted, the tabulation will be as follows :—

Plantaris superficialis medialis,	{	Ramus superficialis medialis.
Plantaris superficialis lateralis,		
Plantaris profundus III,		
Plantaris profundus II,		
Fibulo-tarsalis,	}	Ramus superficialis fibularis.
Plantaris profundus I,		
Interosseus,	}	Ramus profundus.

II. THE CRURAL FLEXORS IN THE LACERTILIA.

A goodly number of papers dealing with the myology of the hind limb of members of the group Lacertilia have appeared, that of Gadow, 82, being one of the most comprehensive. It has been the custom, however, to employ for the various muscles a terminology based upon that used for the mammalia, a procedure which carries with it implications of homologies which in some cases do not exist and in the majority of cases are at best merely partial ones. Since in the present study the lacertilian myology is being approached from below, rather than from above, and since in the amphibia the characteristic feature of the crural muscles is their arrangement in layers, I propose to employ for the reptilian muscles a terminology which will indicate their relations to the amphibian condition, using the terms employed by Gadow, for instance, only for purposes of identification.

A transverse section through about the middle of the crus of *Scincus* presents the appearance shown in Fig. 3. At first sight the differences from the arrangement in *Amblystoma* are very apparent, but a closer inspection will reveal marked similarities, which a study of the nerve supply will but serve to emphasize. The topographical relations of the muscles may, however, first be considered, with a view to determining how far a layered condition can be recognized.

It is a characteristic of the amphibian superficial plantar layer that it arises from the femur and is inserted below into the plantar aponeurosis. In *Scincus* one finds superficially upon the posterior surface of the crus three muscles, a *plantaris superficialis medialis* (*Psm*), a *plantaris superficialis lateralis* (*Psl*), and between the two a long slender muscle which may be termed the *plantaris superficialis tenuis* (*Pst*). Of these the *plantaris superficialis medialis* differs from the other three in that it arises from the head of the tibia, instead of from the femur as might be expected if it be really a portion of the superficial plantar layer. Examining its origin more closely it will be seen to arise not only from the head of the tibia but also from the posterior surface of a strong tendon which passes from the head of the tibia to the internal condyle of the femur. The existence of this tendon and the relation of the muscle

to it is of great importance in determining the true significance of the muscle, for they indicate, apparently, a primary attachment of the muscle to the femur. The tendon, indeed, represents the proximal portion of the muscle which has undergone degeneration in association with a new attachment made by the muscle below the knee joint, the resulting condition being strictly comparable with what has occurred in connection with the peroneus longus of man, this muscle having similarly shifted its upper attachment from the femur to the fibula, its upper part being represented by the external lateral ligament of the knee joint.

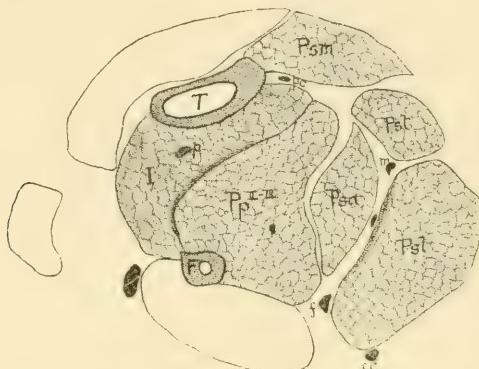


FIG. 3.—Transverse section through the upper part of the crus of *Scincus sp.* *F*, fibula; *f*, ramus superficialis fibularis; *fc*, fibular cutaneous nerve; *I* interosseus; *m*, ramus superficialis medialis; *p*, ramus profundus; *pc*, cutaneous branch from ramus profundus; *PII-III*, plantaris profundus II-III; *Psa*, plantaris superficialis accessorius; *Psl*, plantaris superficialis lateralis; *Psm*, plantaris superficialis medialis; *Pst*, plantaris superficialis tenuis; *T*, tibia.

An examination of the origin of the plantaris superficialis medialis as described for other lacertilia seems to give support to this view. It is true that throughout the reptilia in general the muscle takes its origin from the tibia. In Euprepes, however, Fürbringer, 70, describes it, under the name of the gemellus internus (epitrochleo-tibio-metatarsalis ventralis) as arising both from the head of the tibia and from the internal condyle of the femur, and, according to Gadow, 82, it (gastrocnemius, caput internum) arises in Ophryoessa principally from the posterior surface of the internal condyle, only a few fibres taking origin from the tibia. It is clear then that one is dealing here with a muscle which was either primarily attached to the femur and in the majority of the reptilia has made a secondary connection with the tibia, or else was primarily attached to the tibia and has secondarily migrated, so far as its origin is concerned, upward to the femur.

There seems to be little question but that the former of these two

possibilities is the easier of accomplishment; it is not a migration, but the formation of a new attachment in the course of the muscle and the degeneration of the part above. And, as already noted, there is evidence of the occurrence of such a process in at least one of the muscles found in man. On this view the tibial superficial muscle of the lacertilian crus is to be regarded as having in reality a femoral origin and agrees in its primary relations with the other superficial muscles.

Traced downwards the plantaris superficialis medialis becomes a broad band which inserts into the tibial border of an aponeurotic sheet (Fig. 4, *a*) which represents a portion of the superficial layer of the plantar aponeurosis and receives also the insertion of the plantaris superficialis lateralis.

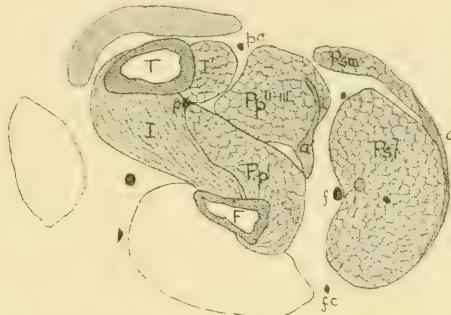


FIG. 4.—Transverse section through the lower part of the crus of *Scincus sp.* *a*, superficial portion of plantar aponeurosis; *a'*, deep portion of plantar aponeurosis; *f*, ramus superficialis fibularis; *F*, fibula; *fc*, fibular cutaneous nerve; *I*, interosseous; *P*, vertical fibers of interosseous; *p*, ramus profundus; *pc*, cutaneous branch of ramus profundus; *PpI*, plantaris profundus I; *PpII-III*, plantaris profundus II-III; *Psl*, plantaris superficialis lateralis; *Psm*, plantaris superficialis medialis; *T*, tibia.

The *plantaris superficialis tenuis* (Fig. 3, *Pst*) takes its origin above the knee joint from a sesamoid cartilage developed in a tendon arising from the fibular border of the flexor tibialis externus (Gadow) and passes downwards to unite with the tibial border of a portion of the plantar aponeurosis which covers the posterior surface of the plantaris profundus II-III (Fig. 4, *a'*). The muscle is slender throughout its entire course. At first it lies superficially between the medial and lateral superficial plantars, but lower down it is covered by the fibular edge of the medialis and at about the middle of the crus fuses with the posterior surface of the deep plantar mass, or, as it is better expressed above, inserts into the tibial border of a portion of the plantar aponeurosis which covers the deep plantar mass.

This muscle does not seem to be present in all lacertilia. Thus Perrin, 93, fails to find it in Uromastix and I have not succeeded in observing

it in dissections of *Phrynosoma*. On the other hand Perrin finds it in *Varanus* and apparently in *Lacerta* and *Gongylus*, and Gadow, 82, regards it as a typical portion of his flexor longus digitorum of which it forms the caput accessorium. It seems to be a muscle separated from the fibular border of the *plantaris superficialis lateralis*, a view which receives confirmation from the statement of Gadow that it is sometimes fused above with that muscle. Its apparent absence in certain forms may upon this view be regarded as due to its failure to separate from the parent muscle.

The *plantaris superficialis lateralis* (Fig. 3, *Psl*) is a rather large muscle which takes its origin by a tendon from the posterior surface of the lateral condyle of the femur. A sesamoid cartilage is imbedded in the tendon just above the line where the muscle fibres begin to make their appearance and, as the tendon is traced downwards, it is found to broaden out into a thin sheet covering the anterior (deep) surface of the muscle and gradually fading out below, with the exception of a narrow band which continues on to the region of the ankle joint, becoming enclosed by the muscle substance (Fig. 4). Just when the tendon begins to fade out an aponeurotic layer (Fig. 4, *a*) appears on the posterior (superficial) surface and increases in strength as it passes downwards, becoming a part of the plantar aponeurosis. It is with the inner border of this that the *plantaris superficialis medialis* and the tendon which descends from the border of the *flexor tibialis externus* and gives rise to the *plantaris superficialis tenuis*, unites.

As the muscle substance is traced downwards it is seen to be continued past the ankle joint into the plantar region of the foot. In the upper part of the muscle the fibers are all parallel, arising from the tendon of origin, but lower down fibers arise from the slender tendon which continues the tendon of origin downwards and have a somewhat radiating arrangement (Fig. 4). Tracing out the two sets of fibers, it is found that the upper ones insert into the upper part of the plantar aponeurosis, while it is those which arise from the prolongation of the tendon of origin that form exclusively the lower part of the muscle and are continued over the tarsus to be inserted into the plantar aponeurosis, and the sesamoid developed in it over the fifth metatarsal.

In addition to these three muscles there is yet a fourth (*Psa*) which is apparently to be reckoned as a portion of the *plantaris superficialis*. It arises from the posterior surface of the external condyle of the femur below the *plantaris superficialis lateralis* and passes downward under cover of that muscle to about the middle of the crus, where it unites with the *plantaris profundus III-II*, or rather, inserts into the portion of the plantar aponeurosis covering that muscle (Fig. 4, *a'*).

Gadow, 82, and Perrin, 93, both describe this muscle as a portion of the flexor longus digitorum (*fléchisseur des quatre premiers doigts*), that is to say of the plantaris profundus III-II, making it a femoral head of that muscle. It may be that it is really a portion of the plantaris profundus group of muscles which has secondarily extended its origin to the femur and that its absence in *Ophryoessa* and *Cnemidophorus*, as noted by Gadow, is due to this upward migration not having taken place. On the other hand it seems more probable that the origin from the femur is a primary condition, the muscle being a separation of the deeper portions of the plantaris superficialis lateralis. Its union with the plantaris profundus presents no more obstacle to this view than the similar union of the superficialis tenuis; both the plantaris profundus and the plantaris superficialis insert primarily into the plantar aponeurosis, so that a union of the two sets of muscles is not at all impossible. On account of its associations with the deep plantar muscles it will be spoken of as the *plantaris superficialis accessorius*.

The plantaris profundus group of muscles is represented in the lacer-tilia by three distinct muscles, one of which is to be regarded as representing the plantares profundi III and II of the amphibian crus, while the other two represent the plantaris profundus I. The *plantaris profundus III-II* (Fig. 4, *Pp^{II-III}*) is the muscle termed by Gadow the flexor longus digitorum, *caput internum*, and by Perrin the *tête interne du fléchisseur des quatre premiers doigts*. It takes its origin from the upper half of the fibula and to a slight extent from the outer surface of the head of the tibia, and increases rapidly in size as it descends the crus, forming the most voluminous muscle of the calf of the leg. At about one-third of the length of the crus an aponeurotic layer appears upon its posterior surface (Fig. 4, *a'*) and into this the plantaris superficialis accessorius and the plantaris superficialis tenuis insert. As it approaches the ankle joint the aponeurosis increases in strength and becomes tendon-like, the fibres of the muscle terminating upon it, and at the ankle joint a sesamoid bone (Fig. 5, *s*) is developed upon its tibial border, the last remaining muscle fibers and also the plantaris profundus I accessorius inserting into this. With the development of the sesamoid bone the whole aponeurosis or tendon becomes thick and almost cartilaginous, but as it is traced onward into the foot it again becomes tendinous and gives off a slip from its fibular border. This passes to the fifth digit, sending off a slip to the fourth, and the main portion of the tendon passes on beneath the superficial muscles of the planta to divide eventually into tendons which pass to the three inner digits. All the five tendons pass to the terminal phalanges of their respective digits and give origin in their course to the lumbrical muscles.

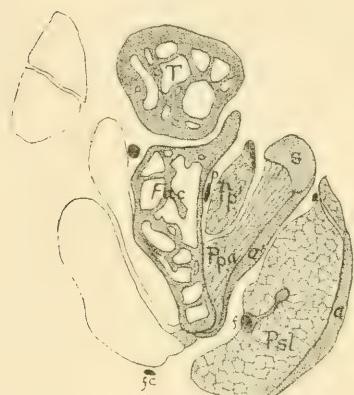
The amphibian *plantaris profundus I* is represented in *Scincus* by two distinct muscles. The first of these, which may be termed simply the *plantaris profundus I* (Figs. 4 and 5, *Pp^I*), arises from the posterior surface of the lower part of the fibula and is directed obliquely downward and inward. It passes over into a flat tendon which lies beneath (anterior to) the tibial border of the tendon of the *plantaris profundus III-II*, and, indeed, is to a certain extent connected to the deep surface of the sesamoid bone developed in that tendon. It separates from it again, however, and is continued on over the large tarsal bone of the first row (astragalo-calcaneus) and is inserted into the two inner bones of the second row of the tarsus. This is the muscle which has very generally been recognized as the *tibialis posticus*.

The second muscle, which may be termed the *plantaris profundus I accessorius* (Fig. 5, *Pp^{Ia}*), arises from the plantar surface of the fibular portion of the large astragalo-calcaneous (*Fac*) and is directed obliquely inward and downward, passing posteriorly to the lower part of the *plantaris profundus I* (*Pp^I*), to be inserted into the sesamoid bone (*s*) developed in the tendon of the *plantaris profundus III-II*.

Finally, there is a well developed *interosseus* muscle (Figs. 3 and 4, *I*) which passes across from the fibula to the tibia, filling up the interval between the two bones through almost its entire length. In the upper part of the muscle (Fig. 3) the fibers have an almost vertical direction, but, as it is traced downwards, the lower fibers, which pass

FIG. 5.—Transverse section through the ankle of *Scincus sp.* *a*, superficial portion of plantar aponeurosis; *a^I*, deep portion of plantar aponeurosis; *f*, ramus superficialis fibularis; *FAC*, fibular-astragalo-calcaneus; *fc*, fibular cutaneous nerve; *p*, ramus profundus; *Pp^I*, plantaris profundus I; *Pp^{Ia}*, plantaris profundus I accessorius; *s*, sesamoid bone in deep portion of plantar aponeurosis; *T*, tibia.

over to the tibia anterior to the higher ones, become more and more oblique, until finally in the lower part of the crus (Fig. 4) all the fibers are exceedingly oblique, some almost transverse, and the vertical upper fibers are seen as a small bundle (*I'*) lying upon the posterior surface of the tibia, completely isolated from the oblique ones. The higher vertical fibers are inserted into the outer (fibular) and posterior surfaces of the lower half of the tibia, while the lower oblique fibers pass to its anterior and inner surfaces, wrapping around the lower end of the bone.



Before passing to a consideration of the nerve-supply of these muscles a few remarks may be made in the way of a comparison of the plantar aponeurosis of the lacertilia with that of the amphibia. In the latter, just as was the case with the palmar aponeurosis, it forms a continuous sheet which receives the insertion of the crural flexors and gives origin to the plantar muscles of the pes, the only indications of a layered condition to be seen in it being at its upper and lower borders, where it becomes partly divided into subjacent layers corresponding to the layers of muscles inserting into or arising from it. In the lacertilia the conditions are slightly different. Covering the posterior surfaces of the plantaris superficialis lateralis there occurs a distinct aponeurotic layer (Figs. 4 and 5, *a*) which receives the insertion of the fibers of that muscle and is also joined by the tendon of the plantaris superficialis medialis. As it is traced downwards this aponeurosis separates in the neighborhood of the ankle joint into a thinner and narrower superficial layer and a thicker and deeper layer. The former gradually verges towards the fibular side as it passes into the foot and is finally lost over the outer side of the fifth metatarsal bone. The deeper layer gives rise from the deep surface of its medial half to the superficial layer of the plantar muscles, while its lateral portion, developing a sesamoid cartilage which receives the insertion of the fibers of the plantaris superficialis lateralis, inserts into the fifth metatarsal.

In addition to this superficial layer a deeper layer of the aponeurosis also occurs (Figs. 4 and 5, *a'*), this being the aponeurosis with which the plantaris profundus III-II becomes connected and which is continued onward as the tendons of that muscle to be inserted into the terminal phalanges of the digits.

There are then in the lacertilia two principal portions of the plantar aponeurosis as compared with the continuous aponeurosis of the amphibia. A deeper portion has separated from a more superficial one to form the tendons of the plantaris III-II and having also inserted into it portions of the plantaris superficialis. Probably too the tendon of insertion of the plantaris profundus I, on account of its attachment to the sesamoid bone developed in the tendon of the plantaris profundus III-II, is to be regarded as a separated portion of the original aponeurosis and, if this be the case, all the crural flexors primarily insert into the plantar aponeurosis as in the amphibia.

Turning now to the nerves of the crus. In sections just above the knee the sciatic nerve is represented by three trunks. One of these (Fig. 6, *A*), when traced onwards, curves around the outer border of the fibula to the dorsal surface of the crus and need not concern us further. The other two are supplied to the flexor surface.

One of them (*F*) passes down into the crus upon the fibular side of the plantaris superficialis lateralis and gives off a couple of large cutaneous branches (*C*) which are distributed to the fibular side of the leg, one of them (Figs. 4 and 5, *fc*) passing far down the crus in the groove

between the fibular border of the plantaris superficialis lateralis and the peroneus. The main stem in its downward course gradually verges medially, so that it comes to lie beneath the plantaris superficialis lateralis (Figs. 4 and 5, *f*) and, indeed, becomes partly enclosed in the substance of that muscle. It appears to give off no branches in the crus, nor could any twig to the plantaris superficialis lateralis be found arising from it. At the ankle it passes into the foot towards its fibular border, and is then recognizable as the external plantar nerve.

This nerve is evidently that referred to by Gadow, 82, as stem III, and, notwithstanding its somewhat different course in its upper part, is apparently equivalent in part to the *ramus superficialis fibularis* of *Amblystoma*, but, unlike it, is quite distinct from the branches which represent the *ramus superficialis medialis*, a difference which may well be correlated with the absence of an anastomosis between the flexor stems in the lacer-tilia.

The other main stem, before leaving the thigh, divides into two trunks (Fig. 6, *m.* and *rp.*), both of which pass downwards to the tibial side of the plantaris superficialis lateralis. The more posterior trunk (*m.*), the stem I of Gadow, may from its distribution be termed the *ramus superficialis medialis*.

Shortly after entering the crus it divides into several branches which are entirely confined to the crus and supply all the portions of the plantaris superficialis, as well as the plantaris profundus III-II.

The deeper branch (*rp.*), the stem II of Gadow, may be termed the *ramus profundus*. It passes towards the tibia and divides into two branches, the posterior of which (Fig. 6, *c*; Figs. 3 and 4, *pc*) is cutaneous and is distributed to the skin over the tibial surface of the crus. The deeper branch enters the substance of the the *m. interosseus* (Fig.

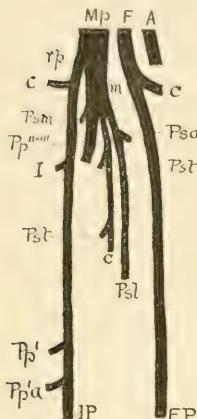


FIG. 6.—Diagram of the crural nerves of *Scincus* sp. *A*, nerve to extensor surface; *C*, cutaneous nerves; *EP*, external plantar; *F*, ramus superficialis fibularis; *I*, branch to interosseous; *IP*, internal plantar; *m*, ramus superficialis medialis; *Mp*, common trunk of ramus superficialis medialis and ramus profundus; *PpI*, branch to plantaris profundus I; *PpIa*, branch to plantaris profundus I accessorius; *PpII-III*, branch to plantaris profundus II-III; *Psa*, branch to plantaris superficialis accessorius; *Psl*, branch to plantaris superficialis lateralis; *Psm*, branch to plantaris superficialis medialis; *Pst*, branch to plantaris superficialis tenuis; *rp*, ramus profundus.

3, *p*) on its posterior surface and is continued downward through that muscle, to which it gives branches, and, emerging from it at the ankle, (Fig. 6, *p*) it sends twigs to the plantaris profundus I and to the plantaris profundus I accessorius and is then continued into the foot as the internal plantar nerve.

Accepting the interpretation of the nerves of the amphibian crus given above and comparing on the basis of their nerve supply the muscles of the amphibian and lacertilian crus the following result is obtained.

Nerve.	Amphibia.	Lacertilia.
R. superf. medialis,	Plantaris superf. medialis, Plantaris superf. lateralis, { Plantaris profundus III, minor, Plantaris profundus III, Plantaris profundus II,	Plantaris superf. medialis. Plantaris superf. tenuis. Plantaris superf. lateralis. { Plantaris superf. accessorius. Plantaris profundus III-II.
R. superf. fibularis,	Fibulo-tarsalis,
R. profundus,	{ Plantaris profundus I, Interosseus,	{ Plantaris profundus I. Plantaris profundus I, accessorius. Interosseus.

It is clear from this that a close comparison based both upon the topographical relations and the nerve supply can be made between the crural flexors of the amphibia and those of the lacertilia, there being, however, in the latter a greater amount of differentiation of the original layers. It is interesting to note that just as in the lacertilian arm no representative of the ulno-carpalis could be distinguished, so too in the crus there appears to be no representative of the amphibian fibulo-tarsalis.

III. THE CRURAL FLEXORS IN THE MAMMALIA.

In considering the crural flexors of the mammalia it will be convenient to depart from the method of description and nomenclature followed in the preceding pages, and to consider the various muscles as independent structures, employing the terms usually assigned to them in mammalian myology. In other words, the primary layers will be temporarily neglected, the reference of the individual muscles to them being considered later on.

A considerable amount of confusion seems to have existed with reference to the soleus and gastrocnemius. Thus, the latter muscle has been described as possessing but a single head in certain forms, the lateral head being described as the soleus; in others the soleus is supposed to be included in the lateral head of the gastrocnemius; and in one case even, the medial head of the latter muscle has been termed

the soleus. It becomes a question then what shall be termed a soleus and what a gastrocnemius, and since the human arrangement is the type with which all other mammalia are directly or indirectly compared, it will be advisable to base the definitions of the two muscles on that arrangement, and this is, essentially, that the gastrocnemius takes its origin from the femur and is a two-joint muscle, while the soleus has its origin from the bones of the crus and is a one-joint muscle. A more satisfactory distinction could be made by referring the two muscles to their respective primary layers, but for the present that given above may suffice.

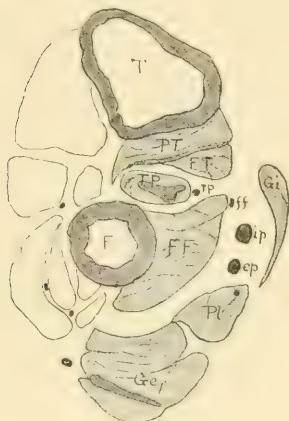


FIG. 7.

FIG. 7.—Transverse section through the lower part of the crus of *Didelphys virginiana*. *ep*, external plantar nerve; *F*, fibula; *FF*, flexor fibularis; *ff*, nerve to flexor fibularis; *FT*, flexor tibialis; *Ge*, gastrocnemius lateralis; *Gi*, gastrocnemius medialis; *ip*, internal plantar nerve; *Pl*, plantaris; *PT*, pronator tibiae; *rp*, ramus profundus; *T*, tibia; *TP*, tibialis posticus.

FIG. 8.—Transverse section through the upper part of the crus of *Didelphys virginiana*. *F*, fibula; *FF*, flexor fibularis; *Ge* and *Ge'*, outer and inner portions of gastrocnemius lateralis; *Gi*, gastrocnemius medialis; *Pl*, plantaris; *PT*, pronator tibiae; *s*, soleus; *T*, tibia; *TP*, tibialis posticus.

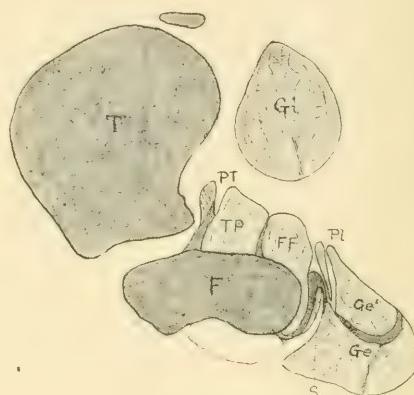


FIG. 8.

The *medial head of the gastrocnemius* is a practically constant element of the mammalian crus and presents little variation except in relative size. In the opossum (Figs. 7 and 8, *Gi*) it arises from the internal condyle and quickly passes over into a flattened tendon which descends the leg, gradually verging toward its outer border, until near the ankle joint it comes to lie to the outer side of the tendon of the gastrocnemius lateralis, in close proximity to which it is inserted into the os calcis. No union occurs between the two tendons except immediately at their insertion. In both the mouse (Fig. 10, *Gi*) and the cat (Fig. 9, *Gi*) the muscle unites with the gastrocnemius lateralis high up in the crus and the conjoined tendon inserts into the os calcis.

In contrast to the extreme simplicity of structure presented by the gastrocnemius medialis is the complexity of the *gastrocnemius lateralis* in all three forms here under consideration. In the opossum the muscle near its origin was found to consist of four bundles. Two of these (Fig. 8, *Ge*) arose close together from the outer surface of the lateral sesamoid cartilage of the knee joint and from the ligament extending from this to the external condyle, and were distinguishable not only by being separated by a band of connective tissue, but also by a difference in the direction of their fibers. A third portion (*Ge^I*) took its origin from the inner (tibial) surface of the lateral sesamoid cartilage, while the fourth portion (*s*) arose from the posterior surface of the head of the fibula, or, to be more precise, from the posterior surface of a tendon which arises from the posterior surface of the head of the fibula and is continued downwards to beyond the middle of the crus upon the deep surface of the compound muscle.

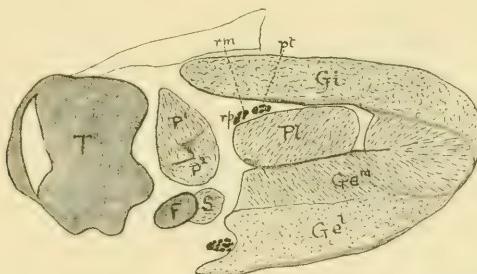


FIG. 9.—Transverse section through the upper part of the crus of the Cat. *F*, fibula; *Ge^l* and *Ge^m*, lateral and medial portions of the gastrocnemius lateralis; *Gi*, gastrocnemius medialis; *P¹* and *P²*, oblique and vertical portions of popliteus; *Pl*, plantaris; *pt*, posterior tibial nerve; *rm*, ramus superficialis medialis; *rp*, ramus profundus; *s*, soleus; *T*, tibia.

Below these four bundles became more or less confused, the connective tissue partition between the portions from the outer and inner surfaces of the sesamoid cartilage persisting for a greater distance than the others, and before its disappearance a tendon appears in the center of the outer sesamoid portions (Fig. 7) and gradually increases in size to become the tendon of the muscle. This is continued down the leg quite independent of the tendon of the gastrocnemius medialis, with which it is inserted into the tuberosity of the os calcis.

In the cat (Fig. 9) the lateral gastrocnemius arises together with the plantaris (*Pl*) from the patella by a strong aponeurotic sheet which is continued backward from the lateral border of that bone, and also from the downward continuation of this sheet which forms an invest-

ment for the outer surface of the plantaris for more than its proximal half. The muscle also takes origin from the lateral surface of the lateral sesamoid bone. The large muscle mass which results is intimately related to the plantaris, and in its upper part a thin sagittal aponeurotic plate appears dividing the muscle into almost equal portions (*Ge^l* and *Ge^m*). This sheet is continued all the way down the leg and below receives the insertion of the muscle fibers, becoming the tendon. In its upper part the muscle comes into contact with the gastrocnemius medialis (*Gi*), an aponeurotic plate intervening between them, however, and below, the tendons of the two muscles fuse completely to be inserted into the tuberosity of the os calcis, the tendon of the soleus also joining them shortly before their insertion, to form a typical tendo Achillis.³

In the mouse I cannot state the exact origin of the muscle (Figs. 10 and 12), but it has essentially the same structure as in the cat and has similar relations with the gastrocnemius medialis and plantaris.

Two other muscles are intimately associated with the gastrocnemius, the one more especially at its origin and the other at its insertion. These are the plantaris and the soleus.

The *plantaris*, notwithstanding its variability in man, is of very constant occurrence throughout the entire mammalian series, and has as a rule a much greater development and a more important rôle than in man. It is always closely associated at its origin with the gastrocnemius lateralis and is inserted below into the plantar fascia (occasionally into the os calcis) by which its action is transmitted to the digits. In the three forms here under consideration it forms what may be regarded as the medial anterior portion of the muscular mass formed by it and the gastrocnemius lateralis. It arises in the opossum (Fig. 8, *Pl*) from the medial half of the posterior surface of a tendon which extends downwards from the lateral fabella, and in the cat, from the fabella and from

³ In sections through the tendo Achillis one sees to the medial side a distinct tendon, connected to the true tendo by thin fascia beneath which lies the tendon of the plantaris as in a groove. This tendon might readily be mistaken for that of the gastrocnemius medialis, but it is in reality a thickening of the crural fascia and is quite independent of the muscle. It is attached below to the os calcis medially to the insertion of the tendo Achillis. Upon the lateral border of the tendo a similar thickening of the crural fascia occurs, but this fuses with the tendon of the gastrocnemius lateralis shortly before it is joined by the soleus. These fascial thickenings have been described for the dog by Ellenberger and Baum, 91, who trace them to the semi-membranous and biceps muscles, but they do not seem to have been noted for the cat, at least they are not mentioned in any of the works on that form to which I have access at present.

the strong aponeurosis which passes backwards from the outer border of the patella. Throughout the greater portion of its extent it is barely separable from the gastrocnemius lateralis (Figs. 9 and 10), but below it becomes tendinous and lies below (*i. e.*, anterior to) and to the inner side of the tendon of the gastrocnemius lateralis, from which, however, it is quite distinct. At the ankle joint it lies to the medial side but posterior to the gastrocnemius and soleus tendon and spreads out into the thin but dense plantar aponeurosis. This covers the insertion of the tendo Achillis or its representatives and passes downward over the tuberosity of the os calcis, being attached to the outer surface of that bone by its outer border, but its inner border and the greater part of its central portion is free. Passing on into the foot it gives rise upon its deeper surface to the flexor brevis minimi digiti and may be continued onward as a series of fascial slips to the bases of the digits.

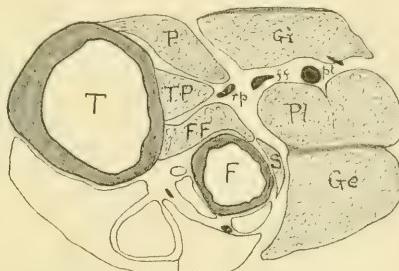


FIG. 10.—Transverse section through the upper part of the crus of the Mouse.
F, fibula; *FF*, flexor fibularis; *ff*, nerve to flexor fibularis; *Ge*, gastrocnemius lateralis; *Gi*, gastrocnemius medialis; *P*, popliteus; *Pl*, plantaris; *pt*, posterior tibial nerve; *rp*, ramus profundus; *S*, soleus; *T*, tibia; *TP*, tibialis posticus.

The important point about the muscle, so far as its insertion is concerned, is its connection with the plantar fascia. That this is its true termination becomes evident in those forms such as *Cuscus* (Cunningham, 81) in which a plate of cartilage is developed in the fascia, the plantaris being inserted into the proximal border of this cartilage. To describe the plantaris as being continuous with the flexor brevis digitorum, as is sometimes done, merely leads to confusion; this muscle really arises from the plantar aponeurosis and the slips which extend to the bases of the digits are also portions of the plantar aponeurosis and have no primary relation to the plantaris.

The *soleus*, unlike the *plantaris* is not always distinguishable in the lower mammals. In both the cat and the mouse (Fig. 11, *s*) it is a well developed muscle which arises from the posterior surface of the upper part of the fibula (Figs. 9 and 10, *s*) and descends the leg beneath the

plantaris and lateral gastrocnemius, eventually becoming tendinous and uniting with the tendon of the latter muscle. In the opossum, however, it does not appear to exist as a distinct muscle and the conditions in this form probably serve to explain its apparent absence in others.

In the description of the gastrocnemius lateralis of the opossum it was noted that it possessed an origin from the head of the fibula. This head seems to be unrepresented in the gastrocnemius of the cat and mouse, and its relations to the rest of the muscle in the opossum present some interesting peculiarities. When it is first seen in tracing a series of sections downward it consists of a thin band of fibers (Fig. 8, s) which arise from a tendon extending downward from the head of the fibula, a portion of the flexor longus digitorum lying between the tendon and the bone. This muscle band is separated at this level from the deeper surfaces of the gastrocnemius lateralis and the plantaris by the tendon which extends downward from the lateral fabella and gives origin to the plantaris. As this tendon gradually fades out below a distinction between the muscle band under consideration and the gastrocnemius lateralis becomes less and less, until, finally, there is complete union of the two.

This fibular head of the opossum seems to represent the soleus of the higher mammalia, and the supposition of Cunningham, 81, and others that the so-called gastrocnemius lateralis of the marsupials includes also the soleus is correct, and the same is probably true of the dog and the other higher mammalia in which the soleus is stated to be lacking. As regards the monotremes it is to be noted that the lateral superficial crural flexor has been termed the soleus, and the gastrocnemius is regarded as lacking (Westling in Leche, 98), this nomenclature being adopted no doubt in view of the fact that the muscle arises from the peculiar process developed upon the upper end of the fibula in these forms and has no connection with the femur. If the fibular process represents a true outgrowth of that bone such a nomenclature would be justified, but it seems really to be an epiphysial structure and in all probability represents the lateral fabella of other forms. On this view the distinction which is made between the marsupial and monotreme muscle practically vanishes and it seems necessary to regard it as representing in both groups the gastrocnemius and soleus of higher forms.

The *long flexors of the digits* in the mammalia have been thoroughly discussed by F. E. Schulze, 66, and by Dobson, 83, and the former has pointed out that the arrangement occurring in man is quite different from that characteristic of the majority of mammals and as a consequence the nomenclature employed in human anatomy cannot be con-

sistently applied in the lower forms. He proposed, accordingly, and his proposition was accepted by Dobson, to speak of the two muscles usually recognized as the flexor longus hallucis and the flexor longus digitorum as the flexor digitorum fibularis and the flexor digitorum tibialis respectively. The proposition is certainly worthy of general acceptance and is almost necessary from the comparative standpoint, since in the majority of mammals the flexor digitorum fibularis (*fl. longus hallucis*) is the principal muscle and the flexor tibialis the subordinate one.

Dobson has pointed out that the relations of the two flexors is according to one of two types and that all the members of any family, if not order, of mammalia will present the same type. In one type the tendons of the two muscles fuse, while in the other they remain distinct, and notwithstanding that he found the aplacental mammalia presenting the second type of relation, Dobson concludes that the first is the more primitive, since, as he states it, "it is difficult to conceive that in any animals in which a definite separation of the tibial from the fibular flexors had once taken place—symmetrical reunion of these tendons could subsequently occur." With such a view the phylogenetic plan here being traced agrees, for an important part of this plan is the recognition of the plantar aponeurosis of the lower forms in the tendons of the long flexors, all the post-axial muscles of the crus, except the interosseus, having their insertion primarily into that aponeurosis, through which their action is extended to the digits.

The descriptions of the long flexors which Dobson has given for so many species of mammals are sufficiently thorough to warrant the omission of a detailed description of the arrangement observed in the forms I have studied, but for the sake of completeness and to bring out especially their relations to the plantar aponeurosis, or rather its mammalian representatives, a description of the arrangement observed in the opossum may be given.

The *flexor fibularis digitorum* (Figs. 7 and 8, *FF*) arises from the inner and posterior surfaces of the greater portion of the fibula. In its upper part it is separated by a strong aponeurosis from the adjacent *tibialis posticus*, and at about the middle of the leg a strong aponeurosis appears upon its posterior surface, separating the muscle from the more superficial *plantaris*. Traced downwards this aponeurosis gives rise upon its posterior surface to a muscle which increases rapidly in breadth, while the aponeurosis diminishes in that dimension, although thickening to form a structure to which the term tendon is applicable. The muscle is the *flexor brevis digitorum*, or rather a considerable portion of it, and need not concern us any further except in so far as its origin from

what is usually described as the tendon of the flexor fibularis serves to confirm the homology of that tendon with part of the plantar aponeurosis of lower forms. Eventually all the fibers of the flexor fibularis insert into the tendon, the last of them disappearing some distance above the ankle joint.

The tendon is continued onward into the foot, lying in the median line between the os calcis and the inner malleolus, and at about the level of the distal row of tarsal bones the tendon of the flexor accessorius passes across it to be attached to its tibial border. This portion of the tendon then separates to pass on to an insertion into the base of the terminal phalanx of the first digit, and later the remainder of the tendon divides into four nearly equal tendons, which pass to the remaining digits. The relations of the lumbricales to the tendons will be considered on another occasion.

The *flexor digitorum tibialis* is, in contrast to the flexor fibularis, a rather slender muscle. It has usually been described as arising from the upper part of the tibia, but in my preparations I have not been able to trace it to that bone. I find it (Fig. 7, *FT*) taking its origin from the strong aponeurosis which covers the posterior surface of the strong pronator tibiae (*PT*), and although it thus comes very close to the upper part of the tibia, no definite connection with that bone could be made out. The difference may be due to the fact that the individuals I studied were advanced fetuses, and that with advancing age the insertion reaches the bone, a process which, if it really occurs, is interesting as denoting a migration of the muscle tibia-wards. Its belly forms an irregularly quadrilateral mass lying between the pronator tibiae internally and the flexor fibularis externally, and resting upon the tibialis posticus. At about the middle of the crus its tendon begins to appear upon its outer surface and into it the muscle fibers gradually insert, until in the lower part of the crus only the tendon remains, resting directly upon that of the tibialis posticus, by which it is separated from the posterior surface of the tibia. At the ankle joint it rests upon the internal malleolus and as it passes onward into the foot it separates from the tibialis posticus tendon and approaches the tendon of the flexor fibularis. At the level of the junction of the proximal and distal rows of the tarsal bones it gives origin to muscle fibers which represent a portion of the flexor brevis digitorum and pass downward and inward to join the rest of that muscle which arises from the tendon of the flexor fibularis.

A little farther on the flexor tibialis tendon becomes connected by fibrous tissue of varying density with the inner border of the flexor

fibularis tendon, but the actual tendon can be traced uninterruptedly onward and, as Dobson states, does not really unite with the flexor fibularis tendon, although the connection between the two may be sufficiently strong as to make a practical union. The tendon then begins to flatten out into a broad band which fades out gradually at the sides into the layer of the plantar aponeurosis with which the plantaris is associated, and eventually associates itself with the flexor brevis hallucis, inserting, in part at least, into the under surface of the cartilaginous spur.

In the other two mammals which I studied the general arrangement of the two flexors was similar to the above, except that the flexor brevis digitorum did not arise from their tendons. The flexor fibularis (Fig. 11, *FF*) is much the larger of the two muscles and sends tendons to all five digits, while the flexor tibialis, in the cat, unites with the tendon of the flexor fibularis before it divides into the terminal tendons. In the mouse the flexor tibialis (Fig. 11, *FT*) arises in common with the tibialis posticus and its tendon remains completely separate from that of the flexor fibularis and fades out into the plantar fascia. In the cat the muscle has an independent origin from the back of the upper part of the tibia.

The *tibialis posticus* has also been described for a large number of forms by Dobson, 83, and I shall indicate only briefly its arrangement in the forms I have studied. In the opossum (Fig. 8, *TP*) it arises from the upper part of the fibula and from a strong aponeurosis which separates it from the adjacent flexor fibularis, and quickly passes over into a tendon which is continued down the leg, under cover of the tendon of the flexor tibialis, and passing behind the inner malleolus is inserted into the scaphoid bone. In the cat it arises from the upper part of the posterior surface of the tibia, becomes tendinous at about the middle of the crus and, passing into the foot in a groove on the inner surface of the tibia, is inserted into the scaphoid. In the mouse (Fig. 11, *TP*) it also arises from the upper posterior part of the tibia as a muscular mass from which later the flexor tibialis separates. It is a slender muscle, soon becoming a tendon and inserting into the internal cuneiform bone.

The *flexor accessorius pedis*, although apparently a muscle of the pes, is considered here with the crural flexors, since its affinities are altogether

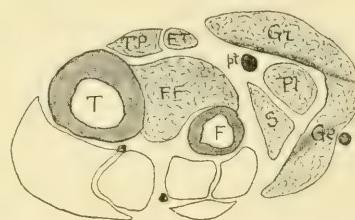


FIG. 11.—Transverse section through about the middle of the crus of the Mouse. *F*, fibula; *FF*, flexor fibularis; *FT*, flexor tibialis; *Ge*, gastrocnemius lateralis; *Gr*, gastrocnemius medialis; *Pl*, plantaris; *Pt*, posterior tibial nerve; *S*, soleus; *T*, tibia; *TP*, tibialis posticus.

with these muscles; the evidence for this statement will be presented later. In the opossum it is a well-developed muscle forming what has been termed by Coues, 72, the flexor brevis pollicis obliquus. Leche, 98, however, records it as wanting in the marsupials and Cunningham, 81, remarks that the muscle is wanting in *Thylacinus* and *Dasyurus*. Dobson, 83, on the other hand, finds in *Dasyurus* a band passing from the *os calcis* to the under surface of the flexor fibularis tendon and identifies it, probably correctly, with the flexor accessorius and Young, 82, in his account of the musculature of *Phascolarctos*, while stating that "there is no flexor accessorius in the foot," goes on to say that a muscular bundle which arises from the *os calcis* and passes to a fibro-cartilaginous backward prolongation of the plantar fascia is regarded by Macalister as similar to it in its nature. McCormick, 87, suggests the identity of one of the heads of his flexor brevis digitorum with the flexor accessorius in *Dasyurus viverrinus*, but the brevity of his description of this head and the absence of explanations of his figures prevent an opinion as to the correctness of the suggestion. There cannot be the slightest question as to the existence of the muscle in *Didelphys virginiana*, and on account of its importance in the fundamental plan of the crural muscles, to be discussed later, it seems quite probable that it may be found in a rudimentary condition in the majority of the marsupials.

In the opossum it arises from the outer surface of the *os calcis* as a distinct bundle of fibers which are directed inwards and distally. They early pass over into a tendon which crosses the plantar surface of the tendon of the flexor fibularis and unites with its outer border, that portion with which it unites immediately separating to form the tendon for the hallux. This description differs somewhat from that of Coues, 72, who regards the tendon of the hallux as representing the direct continuation of the accessorius. Sections show very clearly, however, that this is not the true state of affairs and that the arrangement is as described above. In the cat the accessorius is a strong muscle of considerable size, arising from the outer surface of the *os calcis*. Its thin tendon passes obliquely across the tendon of the flexor fibularis and unites with the greater part of its plantar surface, including the united flexor tibialis tendon. In the mouse it is also well-developed, arising from the outer surface of the *os calcis* and passing obliquely to the tendon of the flexor fibularis, especially to that portion of it which becomes the long flexor tendon of the hallux.

There still remains for consideration the muscle which has been termed the *pronator tibiae* in the monotremes and marsupials and in the higher mammals the *popliteus*, assuming for the present that the two muscles are identical.

In the opossum the pronator tibiale is a muscular sheet which extends obliquely from the fibula to the tibia throughout the greater part of the length of those bones. It takes its origin partly from the inner border of the fibula, but mainly from the strong aponeurosis which separates it from the tibialis posticus and the flexor digitorum tibialis above and the flexor digitorum fibularis below. In its upper part the fibers are directed very obliquely, indeed, almost directly tibia-wards, to the upper part of the tibia, and in this upper portion the muscle is composed of two fairly distinct sheets of fibers, one lying anterior to the other and separated from it by a distinct layer of areolar tissue. Below (Fig. 7, *PT*), however, there is no such separation of two layers, and the fibers have a more vertical course. The partial separation above, already noted by Young, 81, is apparently of "prophetic" interest in fore-

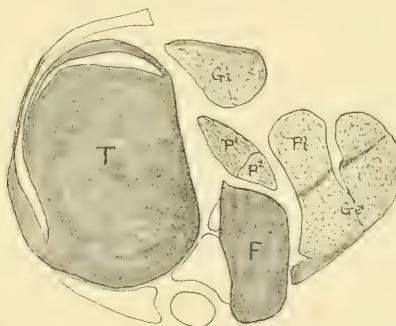


FIG. 12.—Transverse section through the crus of the Mouse just below the knee joint. *F*, fibula; *Ge*, gastrocnemius lateralis; *Gi*, gastrocnemius medialis; *P¹* and *P²*, oblique and vertical portions of popliteus; *Pl*, plantaris; *T*, tibia.

shadowing the differentiation of the muscle into an upper or popliteal portion and a lower or pronator tibial portion.

In the mouse the popliteus arises from a strong fibro-cartilaginous band attached above to the outer condyle of the femur. Those fibers which arise from the tibial side of the band (Fig. 12, *P¹*) have a much more oblique direction than the rest (*P²*) and are inserted into the tibia above them. No distinct indications could be discovered of a representative of the pronator tibiale, *i. e.*, a lower portion of the muscle, although it is possible that some scattered fibers which lie anterior to the main mass of the flexor digitorum fibularis and have an oblique direction, may represent it. A separation between these fibers and the flexor was, however, at best indistinct.

In the cat the popliteus takes its origin from a sesamoid bone which is attached by a strong tendon to the outer condyle of the femur. The

muscle passes obliquely downward and inward over the knee joint (Fig. 9) and shows quite distinctly a composition from two masses of fibers, one of which (P^1), as in the mouse, has an oblique direction, while the other (P^2) is more vertical. No indications of a lower portion of the muscle could be found in the individual I studied, although it may be noted that both in the cat and in the mouse the interosseous membrane is more strongly developed than in the opossum.

IV. THE NERVES OF THE MAMMALIAN CRUS.

In the opossum, at the level where my sections began, there was a main nerve stem, the internal popliteal (Fig. 13), and on one side of it a stem for the internal gastrocnemius (GI) and on the other side

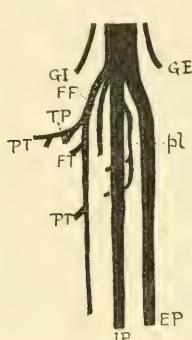


FIG. 13.

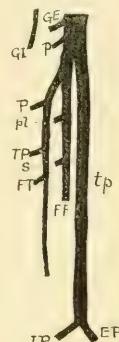


FIG. 14.

FIG. 13.—Diagram of the crural nerves of *Didelphis virginiana*. EP , external plantar; FF , branch to flexor fibularis; FT , branch to flexor tibialis; GE , branch to gastrocnemius lateralis; GI , branch to gastrocnemius medialis; IP , internal plantar; pl , branch to plantaris; PT , branch to pronator tibiae.

FIG. 14.—Diagram of the crural nerves of the Mouse. EP , external plantar; FF , branch to flexor fibularis; FT , branch to flexor tibialis; Ge , branch to gastrocnemius lateralis; Gi , branch to gastrocnemius medialis; IP , internal plantar; P , branches to popliteus; pl , branch to plantaris; s , branch to soleus; TP , branch to tibialis posticus; tp , posterior tibial.

a branch for the external gastrocnemius (and soleus) (GE), and more externally the external popliteal which wound around the head of the fibula to the front of the leg. The internal popliteal descended into the crus between the two gastrocnemii, and soon after divided into five branches, of which two (EP and IP) were quite large, two others were much smaller, one of them (pl) passing exclusively to the plantaris, while the other one (FF) was distributed to the flexor digitorum fibularis.

The fifth branch (Fig. 7, rp) was of moderate size and passed

obliquely inwards, giving off branches to the tibialis posticus (*TP*), the pronator tibiae (*PT*) and the flexor digitorum tibialis (*FT*), and then continued its course downward between the tibialis posticus and the flexor digitorum fibularis, without supplying either, and terminated near the ankle joint, apparently in the periosteum of the lower part of the tibia. The lowest of the branches which passed to the pronator tibiae could be traced downwards in the muscle almost to the ankle joint and seemed to end there in periosteal branches to the lower end of the fibula.

The branch to the plantaris (*pl*) might be described as arising from the internal plantar nerve (*IP*) but with this exception neither the internal nor the external (*EP*) plantar nerves takes any part in the innervation of the muscles of the crus. After they have passed into the foot the external plantar gives off a branch, which passes mainly to the abductor minimi digiti, but also gives two twigs to the flexor digitorum accessorius.

In the mouse (Fig. 14) the internal gastrocnemius is supplied by a branch (*GI*) given off above the level of my highest section. The internal popliteal descends between the two gastrocnemii, gives off branches to the gastrocnemius externus (*GE*) and to the popliteus (*P*), and divides, opposite the knee joint, into three branches, two smaller ones and one large one. The latter (*tp*) is comparable to the posterior tibial nerve of human anatomy in many respects, although it takes no part in the innervation of the crural muscles but descends unbranching to behind the inner malleolus, where it divides into the external and internal plantar nerves (*EP* and *IP*), the former sending a branch to the flexor accessorius.

Of the two smaller branches, one (Fig. 14, *FF*; Fig. 10, *ff*) shortly after its formation gives branches to the plantaris (*pl*) and the soleus (*s*), but passes mainly to the flexor fibularis. The other smaller branch (Fig. 10, *rp*) gives off early in its course a branch to the popliteus (Fig. 14, *P*) and probably supplies the flexor tibialis also, although neither one nor the other of my series of sections permitted of perfect certainty of this point in this form. The branch then descends between the flexor fibularis and the tibialis posticus, giving off a branch to the latter, and, passing more deeply between the two muscles as it descends, finally rests upon the interosseous membrane and seems to terminate in the periosteum of the lower part of the fibula.

In the cat the arrangement of the nerves is in general the same as in the mouse. A branch is given off from the sciatic, before its division, to the internal gastrocnemius and another from the internal popliteal soon after its formation passes to the external gastrocnemius. A little

later the internal popliteal gives branches to the popliteus and to the plantaris, and shortly thereafter divides into two main trunks each of which is composed of subordinate bundles. These two main trunks lie one behind the other (Fig. 9), and the posterior larger one (*pt*) descends the leg without taking any part in the innervation of its muscles and below the ankle divides into the external and internal plantar nerves.

The other trunk is clearly composed of two portions. From one of these (*rm*) branches are distributed to the soleus and to the flexor fibularis, while the other (*rp*) early divides into four branches, one of which is distributed to the popliteus, another to the flexor tibialis, a third to the tibialis posticus, while the fourth, which is very small, passes downward in the aponeurosis between the tibialis posticus and the flexor fibularis, gradually becoming smaller. I was not able to trace this last nerve to its termination, but in all its relations it corresponds to the branch to the periosteum in the mouse.

It may be recalled that in the lower vertebrates the nerves of the flexor surface of the crus were divisible into superficial and deep branches, and that of the former there were two main trunks, one of which, the ramus superficialis medialis, was entirely devoted, so far as its muscular branches were concerned, to the supply of the plantaris superficialis and the plantares profundi III and II. The other superficial trunk, the ramus superficialis fibularis, on the contrary, passed downward, supplying only the fibulo-tarsalis in the amphibia, and became the external plantar nerve. The deep branch, the ramus profundus, was distributed to the plantaris profundi I and the interosseus, and then was continued into the foot to form the internal plantar nerve.

Comparing with this the arrangement described above for the opossum, considerable similarity will be noticed. Thus descending the entire length of the crus there are two nerves, the external and internal plantar, the former of which has practically identical relations with the ramus superficialis fibularis of the lacertilia. In addition there is given off from the internal popliteal at or slightly above its division into the two plantar nerves a smaller stem which supplies the deep muscles of the crus and is continued down to the ankle joint as an exceedingly fine nerve, which is not, however, continued into the foot. In its topographical relations and in its crural muscular distribution this nerve seems to be the homologue of the reptilian ramus profundus, from which, however, it differs in being limited in its distribution to the crus.

In my study of the nerves of the antibrachium (McMurrich, 193) it was shown that the ramus profundus of the amphibia and reptilia extended into the manus, supplying in general the radial part of its palmar

surface, but that in the mammalia its palmar fibers became associated with the median nerve, its antibrachial portion persisting as the anterior interosseous nerve. Apparently a somewhat similar process has taken place in the crus. The tibial plantar fibers have separated themselves from the ramus profundus and have taken a more superficial course to form, in the opossum, the internal plantar nerve, though it can hardly be said that they have united with ramus superficialis medialis, which is represented by the branches to the plantaris soleus and flexor fibularis, together with the branches given off higher up to the two gastrocnemii.

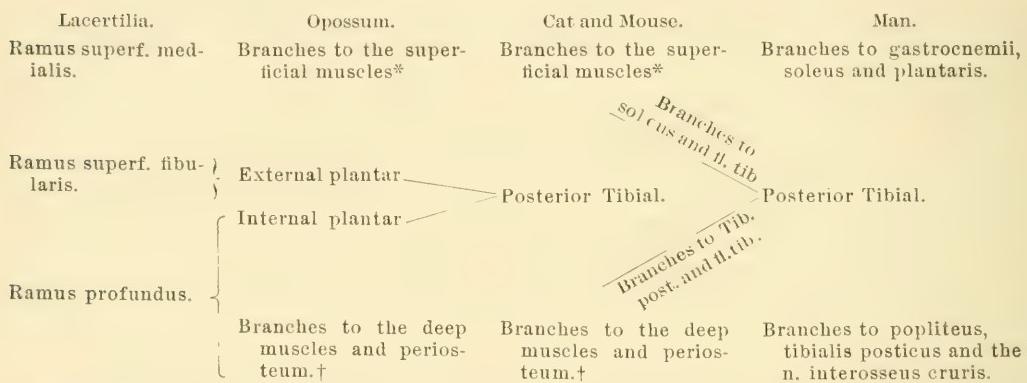
The condition in the opossum does not, however, complete the rearrangement which is characteristic of the mammalia as a group, a further modification consisting in the union of the internal plantar fibers of the marsupial with the ramus superficialis fibularis (external plantar) to form the posterior tibial nerve. It is noteworthy, however, that even although this fused stem appears to be the prolongation of the internal popliteal, yet, in the mouse and cat, the ramus profundus arises from it at the knee joint and that in these forms it is proper to describe the internal popliteal as dividing into the ramus profundus and the posterior tibial, notwithstanding the discrepancy in the sizes of the two nerves. Furthermore the branches for the superficial muscles arise high up, some of them from the internal popliteal before it branches, while others may arise either at the point of bifurcation or even from the upper part of the ramus profundus.

Finally, it may be added, that in man a further modification occurs in the inclusion in the posterior tibial of certain of the fibers of both the ramus superficialis medialis and the ramus profundus, namely, of the former branches to the soleus and to the flexor fibularis, and of the latter a branch to the tibialis posticus and that to the flexor tibialis. Indications of the original conditions are, however, still to be seen in the origin in the popliteal space of a nerve which sends a branch to the popliteus and another to the tibialis posticus and is then continued down the crus, partly in the substance of the interosseous membrane, to end in the neighborhood of the ankle joint. This nerve, whose terminal prolongation down the crus was first thoroughly described by Halbertsma, 47, as the *n. interosseus cruris*, is very evidently equivalent in its topographical relations to the ramus profundus of the lower forms, although some of its fibers destined for the tibialis posticus have separated from it and have joined the posterior tibial nerve. It therefore represents one of the primary branches of the internal popliteal and is deserving of more special mention than is accorded to it in the text-books of human anatomy.

There occurs then in the vertebrate series a progressive modification

of the paths followed by the nerve fibers which supply the flexor muscles of the crus. Stated in general it consists of (1) a separation of the fibers destined for the internal plantar region from the ramus profundus and their assumption of a more superficial course, a process which occurs also in the antibrachium; (2) a breaking up of the ramus superficialis medialis into a number of branches which arise independently; (3) the union of the internal and external plantar nerves to form the posterior tibial; and, finally, the association of some fibers of both the ramus superficialis medialis and the ramus profundus with the posterior tibial.

Taking the reptilian arrangement for a starting point, the rearrangement as it is shown in the opossum, in the mammalia in general as represented by the cat and the mouse, and in man may be schematized thus:—



* By the superficial muscles are here meant the gastrocnemii, plantaris, soleus and flexor fibularis.

† By the deep muscles are here meant the flexor tibialis, tibialis posticus and pronator tibiae (popliteus).

V. THE HOMOLOGIES OF THE MAMMALIAN CRURAL FLEXORS.

Having now described the arrangement of the muscles in the three vertebrate groups selected for study and having also elucidated the modifications presented by the primary nerve stems, we are in a position to determine the homologies of the mammalian muscles with those of the lower forms. A comparison of the lacertilian and amphibian muscles has already been made and the comparison now to be drawn might be principally with the lacertilia, were it not that it will be necessary in the following pages to make frequent reference to the conclusions of Eisler, 95, who deduces the mammalian arrangement directly from the amphibian, neglecting altogether the reptilian. It will be convenient to consider the various muscles in succession and to take the arrangement seen in man as a type.

1. The *gastrocnemius* of man is formed by the union of two heads,

one from the external and the other from the internal condyle, and it unites with the soleus to form the tendo Achillis, inserting into the os calcis. Disregarding the soleus for the present, there are two possibilities to be considered with reference to the double origin of the gastrocnemius; either (1) it represents two originally distinct muscles which have united below, or (2) it represents the splitting in its upper part of an originally single muscle. The second of these possibilities may be dismissed on the ground that in the lower mammals the two heads, as a rule, remain distinct throughout their entire length. Eisler, in accepting the view that the two heads are primarily distinct muscles, takes the ground that one or the other of them has undergone an extensive migration, basing this conclusion upon the crossing of the two tendons which occurs shortly above their insertion, a peculiarity which has been considered in detail by Parsons, 94. The crossing, considered by itself, throws little light upon the question as to which muscle has undergone the supposed migration and Eisler, turning for evidence to the nerve supply, finds that Cunningham, 81, has observed in *Phalangista maculata* that the gastrocnemius medialis is supplied from the external saphenous (sural) nerve, which has a markedly fibular position and he concludes therefore that it is the gastrocnemius medialis which has migrated and that primarily it had its origin from the fibula and lay to the fibular side of the gastrocnemius lateralis, in which case there would be no crossing of the tendons.

The argument by which such a remarkable migration is deduced is open to criticism along several lines. In the first place the crossing of the tendons does not necessarily imply a migration of the muscles. It may be difficult to give a satisfactory explanation of it on another basis, and the migration theory, if correct, would certainly explain it, but it may be pointed out that the same crossing occurs also in the tendons of the flexor fibularis and the flexor tibialis digitorum in man, and yet a reversal of the relative position of the two muscles by migration seems altogether improbable. A theory which explains the one crossing will probably also explain the other, for, it may be noted, the tendons of the gastrocnemius and plantaris represent a superficial layer of the plantar aponeurosis into which both muscles primarily insert, while those of the two long digital flexors represent a deep layer of the same aponeurosis. The most probable factor in the production of the crossing is a physiological rather than a morphological one, a point which will be considered later on in connection with the discussion of the flexor tibialis digitorum.

In the second place it would seem that Eisler has placed too much

stress upon the supply of the gastrocnemius medialis by a branch of the external saphenous nerve. I have not been able to trace the origin of the nerve in the opossum, but one must conclude from Cunningham's statement, 81, that in the thylacine the nerve arises from the internal popliteal. In these two forms then, the thylacine and Phalangista, two different origins of the nerve occur, one of which favors Eisler's migration theory while the other is opposed to it. Which is the more primitive origin? I have not been able to find in the literature accessible to me any sufficiently detailed accounts of the arrangement of the nerves in other marsupials or in the monotremes, but, since there can be no question as to the identity of the lacertilian muscle termed above the plantaris superficialis medialis with the mammalian gastrocnemius internus, the origin of its nerve fibers may throw some light on the question. In *Scincus* it is supplied by a branch from the ramus superficialis medialis, *i. e.*, from the more medial of the two superficial nerve trunks and according to Gadow, 82, this is the usual condition in the lacertilia which he studied, in *Ophryoessa* only does the branch come from the ramus superficialis fibularis. In the crocodiles the muscle is supplied by a branch from the ramus profundus and a weak branch from the superficialis medialis, while in the alligator it receives branches from both superficial nerves, that from the fibularis being the smaller. It seems, therefore, that there is a considerable amount of variation in the course of the nerve fibers in question, a fact which weakens an argument based solely on the path followed by a group of nerve fibers in a single species of mammal.

It seems to me that the muscle in question is primarily and finally a muscle of the tibial side of the crus, and that its homologue in that position can be found from the urodele amphibia to the highest mammalia. Eisler, as has already been pointed out, has failed to recognize the true plantaris superficialis of the amphibia and has thus been led widely astray in his attempts to homologize the amphibian and mammalian muscles. He finds the amphibian homologue of the gastrocnemius medialis in the fibulo-tarsalis (fibulo-plantaris, Eisler) and that of the gastrocnemius lateralis in the plantaris profundus III minor (plantaris superficialis minor, Eisler). It may be pointed out that both these muscles lie beneath the plantaris profundus III (plantaris superficialis major, Eisler) which Eisler identifies with the mammalian plantaris. This latter muscle, however, wherever it can be certainly identified, is in relation with the deeper portion of the gastrocnemius lateralis and would seem to be a derivative of the deeper portion of that muscle. Eisler's identifications would accordingly require an in-

version of the deeper and more superficial muscles, his fibulo-plantaris and plantaris superficialis minor coming to lie on a plane posterior to his plantaris superficialis major. Such a transposition can only be accepted on the strongest evidence, and of this, it seems to me, there is a failure.

Finally, as was pointed out in considering the antibrachial flexors, any theory which requires the migration of a muscle origin over a joint from below demands the closest scrutiny. Eisler's homologies make the gastrocnemius medialis have its origin primarily from the head of the fibula, and to reach the position it has acquired in the lacertilia and mammalia it must have migrated upwards over the knee joint as well as medially. If a plausible homology can be set forth which does not require this migration, the presumption is in its favor. In the forearm it was shown that the palmaris superficialis layer was distinguished from the other flexor layers by having its origin from the humerus, and that throughout the whole series of forms studied it retains that origin. The remarkable similarity which obtains between the amphibian antibrachium and crus leads to the expectation that in all probability the homologue of the superficial palmar layer will have the same relations, and the identification of the plantares superficiales medialis and lateralis with the gastrocnemii exactly fulfills the expectation.

The conclusions to which I have been led, then, are that the gastrocnemius medialis and lateralis of the mammalia are primarily separate muscles which insert into the superficial layer of the plantar aponeurosis, and that they represent the greater part of the superficial plantar layer of the amphibian crus, the gastrocnemius medialis corresponding to the plantaris superficialis medialis of both amphibia and lacertilia and the gastrocnemius lateralis to a portion of the amphibian plantaris superficialis lateralis and to the lacertilian muscle similarly named.

The *plantaris*.—There can be little doubt but that the plantaris is a derivative of the same muscle mass which gives rise to the gastrocnemius lateralis, or, to be more precise, that it represents the deeper medial portion of that mass. For it is typically associated with the gastrocnemius lateralis and is frequently united with that muscle in its upper part, occupying then the position indicated. It is already a distinct muscle in the reptilia, at least the muscle described above as the plantaris superficialis accessorius seems to be its homologue, although the relations which this muscle bears to the plantaris profundus III seems at first sight to preclude any such homology. But it must be remembered that after all the association is not directly with the profundus III, but with the plantar aponeurosis into which the profundus III also inserts.

As a result of the difference in the views of Eisler and myself regarding the amphibian homologues of the gastrocnemii, a difference also exists as to the homologue of the plantaris. Eisler finds it in his plantaris superficialis major, a muscle which, so far as its greater part is concerned, is fibular in origin and has been termed above the plantaris superficialis III. Acceptance of Eisler's homology would again require the migration of a muscle from below over the knee joint and, furthermore, as has already been pointed out, a transition of the planes occupied by the plantaris and the gastrocnemius, both of which phenomena the homology which I have deduced avoids.

It may be added (1) that the primary connection of the plantaris below is with the plantar aponeurosis, its insertion into the os calcis in man being a secondary condition, and (2) that its frequent absence is probably more correctly to be regarded as a failure to separate from the gastrocnemius lateralis, in connection with which idea its not unfrequent union with the tendo Achillis is of significance.

The *soleus*, the third element in the triceps suræ, is a muscle at first sight apparently peculiar to the mammalia, and among these is possibly unrepresented as a distinct muscle in the monotremes.⁵ It has been described as lacking in a number of mammals, in such cases being probably included in the gastrocnemius lateralis. It has characteristically an origin from the fibula and this points strongly to its being a representative of the plantaris profundus group of muscles. The conditions in the lacertilia throw little light upon the question, but it is to be noted that the two superficial profundus layers are fused together in these forms. They are, however, clearly distinguishable in the amphibia and it is possible that they again become separated in the mammalia, a series of modifications similar to those which occur in the antibrachial flexors taking place. If this supposition be correct then it seems probable that the soleus represents the plantaris profundus III of the amphibia. The forms which I have studied do not furnish sufficient data for certainty as to this homology, but it seems to be the only one consonant with the facts at our disposal. Possibly a renewed study of the monotreme erus with this idea in mind may yield some light. Eisler, it may be added, regards the soleus as a derivative of the gastrocnemius lateralis.

The *flexor fibularis* and the *flexor tibialis* are so closely associated that at first one would have little hesitation in assigning them to a common

⁵The erroneous application of the term soleus to the muscle which arises from the epiphysial process of the fibula has already been noted.

origin, the two muscles standing to one another in much the same relation as the flexor profundus digitorum and the flexor longus hallucis of man. Their nerve supply is altogether different, however, since the flexor fibularis is supplied by the equivalent of the ramus superficialis medialis, while the flexor tibialis is supplied from the ramus profundus. There seems to be no good reason why this should be so if the two muscles belong to the same original layer, and one is forced to the conclusion that they have their origin from quite different layers. In the amphibia and lacertilia it has been shown that the plantares profundi III and II are supplied from the ramus superficialis medialis, while the plantaris profundus I and the interosseus are supplied from the ramus profundus. The flexor fibularis, accordingly, probably represents the plantaris profundus II, if the soleus be regarded as equivalent to the plantaris profundus III, while the flexor tibialis probably represents in part the plantaris profundus I. In other words the flexor tibialis is a muscle derived from the same primary layer as the tibialis posticus and is quite distinct from the flexor fibularis.

This view may seem improbable on account of the close relation of the two muscles in their lower portions and on account of the distinctness of the tibialis posticus, but it must be remembered that the primary insertion of a considerable portion of the plantaris profundus I is probably into the plantar aponeurosis and that in the lacertilia it is in part united to the sesamoid cartilage developed in the tendon of the plantaris superficialis III-II. It is this aponeurotic portion of the profundus I which becomes the flexor tibialis, while the remainder of it constitutes the tibialis posticus, and, as will be shown later, the flexor accessorius digitorum pedis.

Eisler finds the homologue of the flexor tibialis in his plantaris profundus II and that of the flexor fibularis in his plantaris profundus I, thus coinciding with the opinion expressed above that the muscles belong to different primary layers and also with the identification of the two muscles, since the muscle he names the plantaris profundus I is identical with that which I have called the plantaris profundus II and that which he calls the plantaris profundus II is a part of my plantaris profundus I.

The remarkable transference of the action of the flexor fibularis from the fibular digits to the great toe which occurs in the mammalian series has received its most plausible explanation from Keith, 94, on functional grounds. It remains to especially emphasize in connection with his argument the primary insertion of both muscles into the deeper layers of the plantar aponeurosis, the different arrangements of the tendons

of the two muscles being but various differentiations, due to differences of strain, of an originally single aponeurosis and not a secondary fusion of distinct structures.

The significance of the *tibialis posticus* has already been indicated; from its relations and nerve supply it seems unquestionably a derivative of the *plantaris profundus I*, a view not at variance with that of Eisler, when allowance is made for the differences in our terminologies. Another derivative of the same layer is the *flexor accessorius digitorum pedis (quadratus plantæ)*, which represents a portion of the layer which takes its origin from the tarsal bones and is inserted into the plantar aponeurosis. The muscle certainly finds no place in the general plan of the plantar muscles and is clearly represented in the lacertilia, where it is supplied by a branch from the ramus profundus. Its supply from the external plantar nerve in the mammalia is readily explained on the basis of the separation of the plantar fibers from the ramus profundus to form a special more superficial nerve stem and to subsequently unite with the external plantar fibers to form the posterior tibial nerve, as has already been described. Its relations to the tendon of the long digital flexor is clearly a persistence of its original insertion into the deep layer of the plantar aponeurosis.

Finally as regards the *popliteus*, the most usually propounded homology is with the uppermost portion of the *interosseus* muscle, and, in truth, at first sight this seems to be a most plausible suggestion. There are, however, some difficulties in its way, one of the most important being its origin from the external condyle of the femur and another that in some forms it covers in, *i. e.*, lies posterior to the upper portions of the *flexor tibialis* and the *tibialis posticus*. On the other hand, its constant supply from the ramus profundus seems to imply in almost unmistakable terms its derivation from either the *interosseus* or the *plantaris profundus I*, and of the two the *interosseus* seems to be its most likely origin.

Eisler, though, recognizing a possibility of referring it to the *interosseus*, finally concludes that it is not properly a crural muscle at all in the sense in which the term crural is used here, but that it is a femoral muscle and the equivalent of the *brachialis anticus* of the arm. It is difficult to see how such an homology can be worked out in its details. It would imply that the muscle is a derivative of one of the femoral flexors, most presumably of the *biceps* or better of such a muscle as the *eructo-coccygeus* of the opossum, which sends a slip obliquely across the thigh to be inserted into the shaft of the tibia. It is to be noted, however, that this slip passes superficially to the upper part of the *gastrocnemius*, while the *popliteus* passes beneath, *i. e.*, anterior to that muscle.

The opossum has no muscle which corresponds exactly to the popliteus. It has the homologue of the interosseus well developed as the pronator tibiae, but that muscle is entirely confined in its origin to the fibula, even its uppermost portion which has been homologized with the popliteus arising from that bone. It is only in the higher forms that a true popliteus is found and certain peculiarities in its structure in the mouse and cat seem to throw some light upon its significance. In both these forms, as has already been noted, two very distinct portions can be discerned in the muscle, a more tibial portion whose fibers have a very oblique direction and a more fibular portion whose fibers are more nearly vertical. A distinct line of demarcation between the two parts occurs in any transverse section of the upper part of the crus. Furthermore, the muscle receives two nerves, a fact which in so small a muscle is in itself noteworthy, and is all the more significant in that one of these nerves arises, in the mouse for instance, with that for the soleus from the internal popliteal stem, while the other arises from the branch which I have identified with the crural portion of the ramus profundus of the lower vertebrates. And, finally, the internal popliteal branch is supplied entirely to the more tibial oblique-fibred portion of the muscle, while that from the profundus passes entirely to the more fibular vertical portion.

The significance of these facts seems to be evident. The popliteus is a compound muscle, consisting of a portion derived from the plantaris superficialis and a portion which represents a part of the pronator tibiae of the marsupials and the interosseus of the lower vertebrates. In other words the constitution of the mammalian popliteus is exactly equivalent to that of the pronator radii teres in the arm.

The idea that the muscle is a composite one furnishes a simple explanation of the condition occurring in some carnivores. Gruber, 78, has shown that in the dog, wolf and fox there exists, independently of the popliteus and lying to a certain extent beneath it, a short muscle extending between the upper portions of the fibula and tibia. This is the *m. peroneo-tibialis*. The same muscle occurs also in Viverra (Dobson, 83), and as an anomaly in man (Gruber, 77 and 78). The fact of the occurrence of such a muscle in certain carnivores while lacking in others is certainly reasonably accounted for on the supposition that its absence in the latter is only an apparent one. That is to say, it seems probable that the peroneo-tibialis of the dog represents the more vertical portion of the popliteus of the cat, the dog's popliteus being equivalent to the obliquely fibered portion of the cat's muscle. And similarly, the appearance of the peroneo-tibialis as an anomaly in man may readily be ex-

plained on the ground of a separation of the profundus portion of the popliteus from the superficialis portion.

In the opossum the upper partially separated portion of the pronator tibiae is very probably the equivalent of the peroneo-tibialis element, but what may be the representative of the superficialis portion of the popliteus it is difficult to say. A possible degenerated representative of it may be found in a strong tendon-like band which extends obliquely across the knee joint from the external fabellar cartilage to the head of the tibia, but such an identification can be at present merely a suggestion. More important, perhaps, are the relations which seem to exist between the plantaris and the popliteus as shown by anomalies in man, the popliteus having occasionally an accessory head which often coincides with the absence of the plantaris.

If the tendon mentioned above as occurring in the opossum really prove to be the representative in that animal of the superficialis portion of the popliteus, then there should be some muscular representative of it in lower forms. This may be found in that portion of the monotreme popliteus which arises from the epiphyseal process of the fibula, and in the reptilia it may have a representative in the plantaris superficialis tenuis, although this seems at present very questionable. A study of a greater number of forms than I have had at my disposal will be necessary to trace out all the homologies of the popliteus, but I believe that the observations here recorded make the supposition as to the composite nature of the popliteus exceedingly probable.

In conclusion a few words may be said with regard to the modifications and homologies of the *plantar aponeurosis* throughout the series. In the urodele amphibia it is represented in the crus by the aponeurosis which covers the posterior surface of the plantaris profundus III and by the tendons of the plantares superficiales medialis and lateralis. It receives, therefore the insertions of these three muscles, together with that of the profundus II and a part of that of the profundus I, and gives origin to the superficial muscles of the plantar surface of the foot. With the increase in size of the lateral and medial portions of the plantaris superficialis, a portion of the superficial layer of the aponeurosis becomes separated to form the tendon of those muscles, while the rest of it is covered in by them and remains included in the tendon of the plantaris profundus III-II, part of it giving insertion to the plantares superficiales accessorius and tenuis.

This is the reptilian condition, and the transition from it to that of the mammalia is comparatively simple. The superficial layer of the aponeurosis in the mammalia is represented by (1) the tendon or tendons

of the triceps suræ, (2) the tendon of the plantaris and (3) the plantar aponeurosis of the foot, a portion of it (4), however, remaining included with the deep layer in the tendons of the flexores fibularis and tibialis. It is this fourth portion of the superficial layer which gives origin to the flexor brevis digitorum in those forms in which that muscle arises from the tendons of the long flexors.

The homologies of the crural muscles traced out in the preceding pages may be tabulated thus:

Amphibia.	Lacertilia.	Opossum.	Mammalia.
Plantaris sup. medialis.	Plantaris sup. medialis.	Gastrocnemius medialis.	Gastrocnemius medialis.
Plantaris sup. lateralis.	Plantaris sup. lateralis. Plantaris sup. accessorius. Plantaris sup. tenuis.	Gastrocnemius lateralis (less included soleus). Plantaris.	Gastrocnemius lateralis. Plantaris. Popliteus (superficial portion)? Soleus.
Plantaris prof. III.	Plantaris prof. III-II.	Soleus portion of gas- troc. lat.	Flexor fibularis.
Plantaris prof. II.		Flexor fibularis.	Flexor tibialis.
Plantaris prof. I.	Plantaris prof. I.	Flexor tibialis.	Tibialis posticus.
Interosseus.	Plantaris prof. I access. Interosseus.	Tibialis posticus. Flexor accessorius. Pronator tibiae.	Flexor accessorius. Popliteus (peroneo-tibial portion).

SUMMARY.

1. In the crus of the urodelous amphibia the flexor muscles are arranged in five layers, the superficial one arising in the femoral region, the others, which have a more or less oblique direction, taking their origin from the fibula and slightly from the tarsus. They are inserted for the most part into the plantar aponeurosis, only the deepest layer inserting into the tibia. Between the second and third layers is a slender longitudinal muscle extending between the fibula and the tarsus.

2. The nerves of the flexor muscles of the amphibian crus are arranged in two main trunks, a ramus superficialis and a ramus profundus. The latter is continued into the pes as the internal plantar nerve. The former divides into rami mediales which are confined to the crus and a ramus fibularis which is continued into the pes as the external plantar nerve. The rami superficiales mediales supply the first, second and third layers of muscles, the ramus superficialis fibularis, the fibulo-tarsalis and the ramus profundus the fourth and fifth layers.

3. A complete separation of the præaxial and postaxial nerve fibers does not take place at the knee-joint in the amphibia, but the ramus profundus for a considerable portion of its course contains fibers which are distributed to the præaxial surface of the crus.

4. In the lacertilian crus the same muscle layers that occur in the

amphibia are readily distinguishable. The superficial layer has increased greatly in size and shows a differentiation into several muscles. The second and third layers have fused and the fourth layer has differentiated into two separate muscles. The fibulo-tarsalis has disappeared and the muscles have in general a more vertical direction than in the amphibia.

5. The arrangement of the nerve trunks in the lacertilian crus is essentially the same as in the amphibia. The separation of the *præaxial* and *postaxial* fibers takes place, however, above the knee joint.

6. In the mammalia the same layers of muscles can be distinguished although they have undergone greater differentiation into individual muscles than in the lower forms.

7. The plantar fibers of the ramus profundus are separated in the mammalia from the crural fibers and in the opossum form a more superficial stem, the internal plantar, which traverses the crus without taking part in its nerve supply. The other rami remain practically unaltered. In the higher mammalia a further change takes place in that the ramus fibularis (external plantar) and the internal plantar unite to form a single stem, the posterior tibial, and, in man, some of the fibers belonging to the ramus superficialis mediales and the ramus profundus become included in this.

8. The superficial layer of muscles retains throughout its origin from the femur and the deep layers theirs from the crural bones, with one apparent exception. Furthermore the insertion into the plantar aponeurosis is largely retained, although some shifting to the bones occurs.

9. The soleus represents the second layer of muscles and its absence in certain forms is probably due to its inclusion in the gastrocnemius lateralis.

10. The flexor fibularis and flexor tibialis belong to different layers, the former representing the third layer, while the latter is formed from a portion of the fourth layer, as is also the tibialis posticus.

11. The flexor accessorius digitorum (*quadratus plantæ*) is primarily one of the crural muscles and represents another portion of the fourth layer of muscles.

12. The popliteus is a compound muscle, being formed of a portion from the superficial layer, united with a portion of the fifth layer. The occasional occurrence of a distinct *m. peroneo-tibialis* in the higher mammalia is probably due to a failure of the two portions to unite.

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THE FRAMEWORK OF THE GLANDULA PARATHYROIDEA.

BY

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WITH 3 TEXT FIGURES.

In studying the framework of the thyroid gland in man and the higher mammals, the author was enabled at the same time to make certain observations concerning the supporting tissue of the glandula parathyroidea. These investigations were carried on by means of the destructive digestive methods through which the cytoplasmic elements are all dissolved, leaving the resistant framework of the organ in the form of an opaque skeleton, which reveals its original form and relationships, and demonstrates clearly at the same time, the course of the various interstitial processes in three dimensions of space. The details of the method have already been published in another place.¹ In both the monkey and the dog the parathyroid bodies are situated within the general capsule of the glandula thyroidea. Under normal conditions in both the living organ and in fixed tissues, the small oval gland is scarcely elevated from the surface of the larger organ in which it is contained. The fasciculated capsule of the thyroid practically splits and embraces the gl. parathyroidea which is oval in both longitudinal and transverse dimensions. Accordingly the capsule of the thyroid becomes the capsule of the parathyroidea, with no essential differences in structure. Like the capsules of most organs it is composed of laminated fasciculi of white fibrous tissue, with a considerable amount of reticulum in its inner surface. This capsule contains a small amount of elastic tissue, some of which may accompany the larger septa that follow the greater vessels into the substance of the gland. In piece digestions which have been cut through the thyroid and parathyroid, the organ is clearly shown in three dimensions. In both dog and monkey, the parathyroid is $2\frac{1}{2}$ mm. broad, about 4 mm. long, and about 2 mm. in thickness. When viewed with a stereoscopic microscope, the organ is seen just within the capsule

¹ Flint: Bulletin of the Johns Hopkins Hospital, 1901; Arch. f. Anat. u. Ent. Anat. Abth., 1903.

of the gl. Thyoidea where its finer structure and limiting envelope bring it out in sharp contrast to the follicles of the thyroid that embrace it on three sides. The little organ is oval in both transverse and longitudinal planes giving it plastically the form of a prolate spheroid. As shown by this method the structure of the thyroid has been previously described² and when the plane of section includes both organs, a glance is sufficient to separate them owing to the marked differences in their structure. At first sight in piece digestions, the parathyroid has a homogeneous, ground-glass appearance without showing any very striking features excepting the blood vessels that traverse its substance; but if the specimen is carefully studied with high oculars and rapid alterations in the quantity and

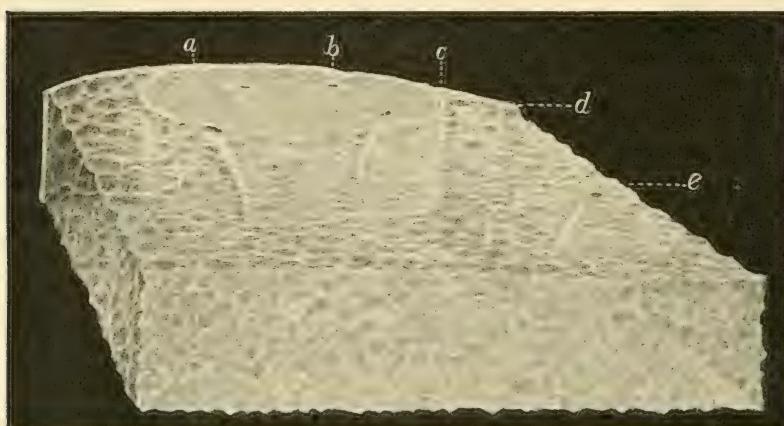


FIG. 1.—Picce Digestion of the Thyroid and Parathyroid of a Monkey. Extracted with ether, digested with trypsin and cleared in glycerine. $\times 19$. The general form and arrangement of the thyroid follicles are readily made out. Beneath the capsule and embraced by its split laminae is the parathyroid body. The large septa and vessels as well as the finer septa can be seen on the surface, while the vessels are readily followed into the depths.

a=Finer septa of parathyroid. b=Blood-vessel and coarser septa. c=Capsule of parathyroid at point of splitting. d=Capsule of thyroid. e=Follicles of thyroid.

variety of light with which it is illuminated, delicate, fine septa on the surface of the organ come into view. Owing, however, to their extreme delicacy they are indistinctly shown and the picture accordingly is not as instructive as one from those organs where the connective tissue is accumulated into larger and more definite processes and septa.

Besides the more delicate septa that embrace the cell complexes of the parathyroid we see the blood vessels which are always accompanied by relatively thick connective tissue processes. As a rule these run in the

² Flint: The Johns Hopkins Hospital Bulletin, 1903.

central portions of the gland although instances are not uncommon where they are found either in the capsule or its neighborhood indicating their points of entrance and exit to and from the substance of the organ. The quantity of the connective tissue diminishes with the order of ramification until the small arteries, veins and capillaries are reached. These naturally are found in the finer septa or trabeculae about the cell columns.

To Ludwig and his pupils we owe the view that many organs are divided into a series of similar structural units which have constant and definite relations to connective tissue processes, blood vessels, nerves and lymphatics. An organ is composed of a great many of such units which are repeated again and again in its formation. Glands like the pancreas, salivary gland, liver, and spleen express their structural relationships excellently while others as for example the stomach and adrenal cannot

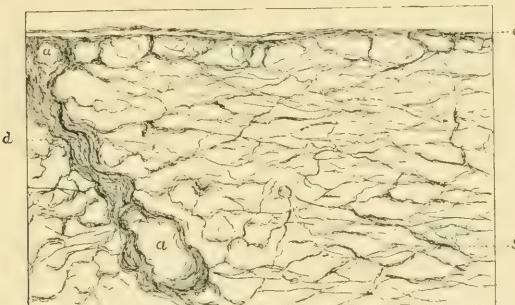


FIG. 2.—Section of block of tissue shown in Fig. 1, about 45 microns thick. $\times 37$. Stained with Aniline blue. Drawn over a blue print which was subsequently washed out. The section shows the capsule of the parathyroid, the larger septa and blood-vessels as well as the smaller septa limiting the cell columns. *c*=Capsule. *s*=Finer septa. *d*=Coarser septa. *a*=Blood-vessel.

be subdivided at all. Accordingly when we know the finer structure of one unit we know the structure of the whole organ with the exception of the relation of these units to each other. In this sense, however, there are no structural units in the parathyroid which bear a constant relationship to connective tissue processes. The ultimate structural integers must be looked upon as the cell columns or cell groups and the adjacent fibrous tissue which supports them.

In thin digested sections the framework appears as irregular septa which do not form a continuous network throughout the organ, but are broken up into smaller processes which support the irregular coiled columns of cells of which the organ is composed. These septa carry the arteries, capillaries, veins, and nerves. They are in some places built up of fasciculi of reticulum fibrils, in others, of a thinner, looser formation

of anastomosing and branching fibrils. When thick, stained, digested sections from 50 microns up are studied, these broken septa are obviously continuous in the third dimension with other processes that turn off and occupy various planes according to the branching of the anastomosing cell columns. In this way in these preparations, especially under the low power, it often appears as though the framework formed a continuum stretching across the gland. In considering the structure and arrangement of the framework in three dimensions, this is, of course, true, the broken irregular septa appearing only in thin sections where the con-

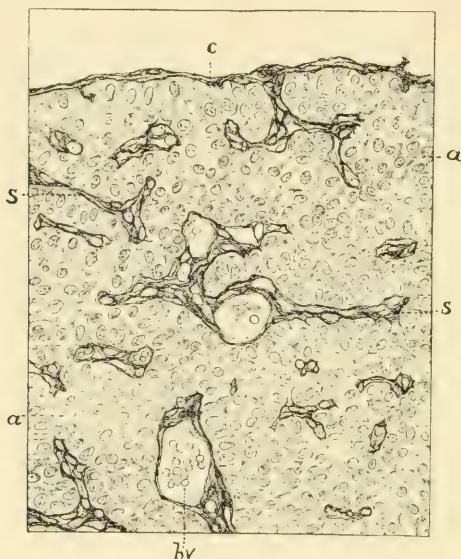


FIG. 3.—Section of parathyroid of dog. Stained by Mallory's method. $\times 62$. c=Cap-sules. s=Finer septa. a=Cell columns. bv=Blood-vessel.

tinuity of the third dimension is broken. Under ordinary circumstances the septa are comparatively fine and delicate. Relatively speaking, however, the framework is not abundant. Occasionally large septa project inwards from the capsule, either in connection with or independent of the blood vessels. The majority of the large vascular trunks, however, as is seen in piece digestions also, are found in the center of the organ. Around the adventitia, the framework is abundant and in these situations large fasciculi of considerable dimensions are often found. When thin sections are studied under the immersion lens, the framework can readily be resolved into the ultimate constituent fibrils of which it is composed. These fibrils branch and anastomose, and are of extreme delicacy. In

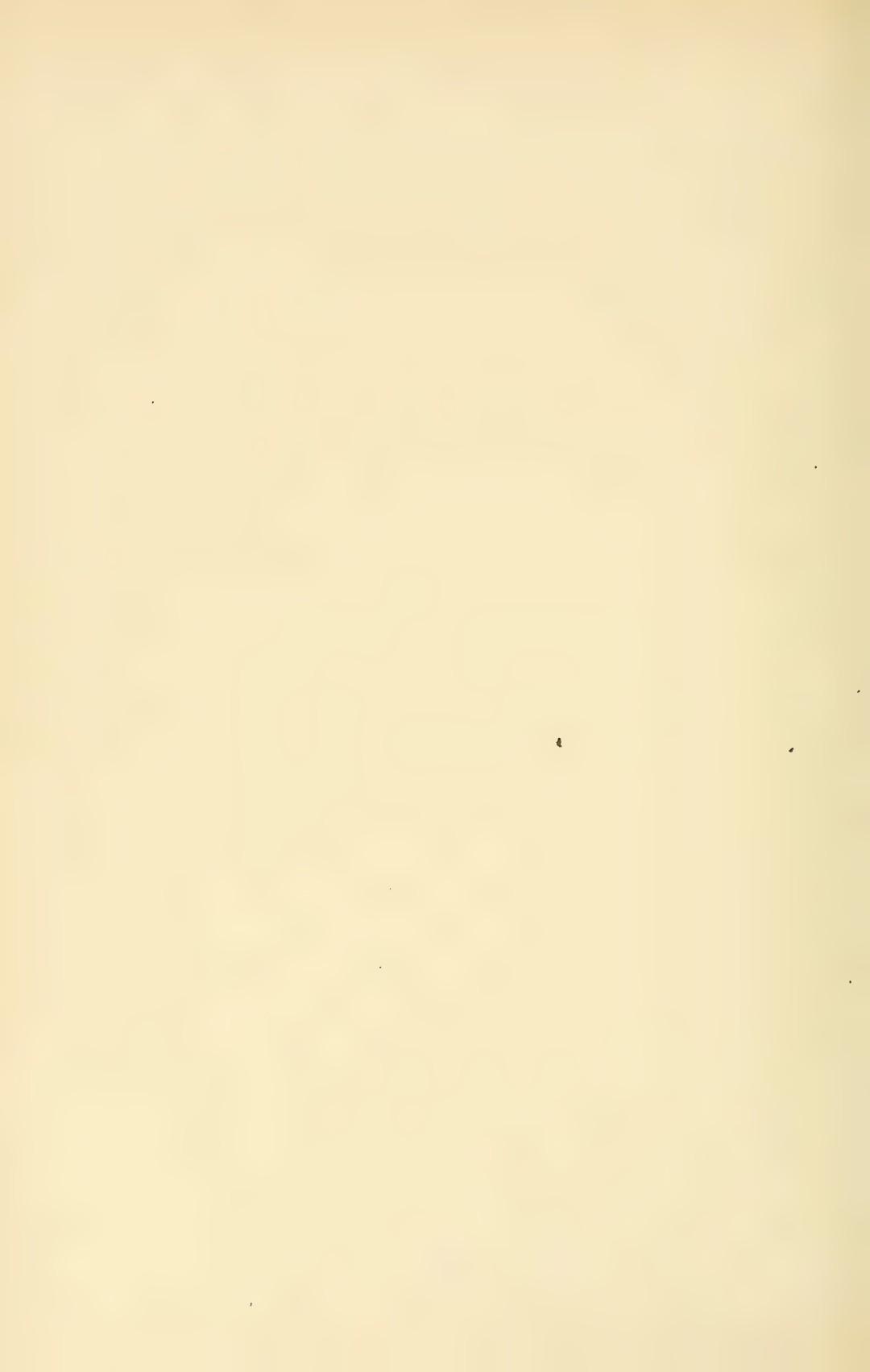
sections stained by the ordinary methods and thin sections varying from 3 to 6 microns in thickness, stained by Mallory's connective tissue stain, numerous cells with oval nuclei are found embedded in the fibrils. These are the connective tissue corpuscles, and do not differ in this position from those found in other parts of the body. In Mallory specimens (Fig. 3) the relationship of parenchymatous cells to the connective tissue processes are clearly shown. The cells are polygonal in shape, composed of granular cytoplasm which stains readily with the acid dyes. These cells contain spherical nuclei of medium size, possessing a well marked nuclear membrane with a considerable amount of chromatin along the linin filaments. They are packed together in irregular coiling and anastomosing columns (Fig. 3, *a*) of varying size. In some instances as many as seven or eight cells may be interposed between septa and blood-vessels while in others only two or three are so placed. No connective tissue fibrils pass in between the cells to form a finer framework. They rest against each other, and are supported by the adjacent septa.

RÉSUMÉ.

(1) In piece digestions, the gl. parathyroidea of the dog and monkey is seen in the form of a prolate spheroid embraced by a capsule formed through a splitting of the capsule of the thyroid gland. Within the gland the larger connective tissue processes accompanying the blood-vessels are easily seen usually in the central portion of the organ which under the low powers of the stereoscopic microscope has a homogeneous ground-glass appearance. Under the higher powers, however, the delicate septa embracing the cell columns can just be made out.

(2) In thin stained digested specimens, the framework appears as irregular broken septa composed of anastomosing and branching fibrils as well as fasciculi or bundles of fibrils. These septa support the irregular anastomosing cell columns of which the gland is composed. In thick, stained, digested specimens, however, septa can be followed in three dimensions where they give almost the appearance of a closed network owing to the change of direction as they follow the cell complexes of the gland in the depths of the section.

(3) The relations of the cells to the connective tissue as shown in these sections, indicates that the cell columns are supported by the septa. Fibrils from the septa do not run in between the individual cells. The cell columns are irregular in thickness, and anastomose with each other. The smaller vessels are found in the smaller septa.



THE DEVELOPMENT OF THE CRANIAL AND SPINAL
NERVES IN THE OCCIPITAL REGION OF THE
HUMAN EMBRYO.

BY

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WITH 4 PLATES AND 14 TEXT FIGURES.

The following paper reports the results of a study of the morphology of the ninth, tenth, eleventh, and twelfth cranial and the upper cervical nerves, together with their ganglia, in a series of human embryos. It includes a description of all the stages in the development of these structures from the time at which they can first be definitely outlined from the surrounding mesodermal tissue up to the time they have reached adult conditions. This work was made possible through the kindness of Prof. O. Hertwig, Prof. His, and Prof. Mall, who gave the writer access to their valuable embryo collections for the purposes of this study. For this courtesy the writer takes advantage of the present opportunity to express his appreciation. Acknowledgment is also to be made to Prof. Gage, whose Buxton embryo is included in the series studied.

The ultimate histogenesis of the nerve elements, a question which has recently been thoroughly gone over by *Harrison*, 01, and *Bardeen*, 03, will not be taken up. In the earliest stage where reconstruction was possible the right and left divisions of the ganglion crest have migrated ventro-laterally along the side of the neural tube, and are about to form secondary attachments to it. Fibroblast formation is at this time well under way, and peripheral fibre paths are beginning to become definite. It is the consideration of the size, form, and relation of these paths and the associated ganglion cell masses, in their different stages of growth, toward which attention has been directed.

The results of this study are tabulated at the end of the paper; but special mention should be made of the eleventh cranial nerve. In tracing out its early history it becomes more than ever apparent that it is absolutely similar and continuous with the tenth or vagus nerve. In the embryo these exist, not as two independent cranial nerves, but rather as parts of a single structure, each part possessing mixed motor and

sensory roots with root ganglia derived from the same ganglion crest. As the development progresses the cranial end of this complex becomes predominantly sensory and the caudal end predominantly motor, and also more spread out which gives rise to a difference in the appearance of the two portions in the adult, and has resulted in their being considered as two independent structures.

That such a relation between the tenth and eleventh cranial nerves exists is not a new idea, but was long ago suggested by the work of *His*, 88, on the human embryo, though this investigator did not work with sufficiently young stages to make the evidence conclusive. The theory has since then been supported by the work of *Fürbringer*, 97, and *Lubosch*, 99, who believe that phylogenetically the tenth and eleventh nerves cannot be separated. *Chiariugi*, 90, however, from the comparative embryology of these structures, concludes that the eleventh is not a part of the tenth, but is a nerve for itself which results from the differentiation of the nucleus of origin of the ventral roots into median and lateral divisions; the latter rootlets losing their segmental distribution take a new course and depart obliquely through the cranium as an independent nerve. Another view regarding these nerves is offered by *Minot*, 92. He suggests that a modification may have occurred in the motor fibres of the dorsal roots of the hypoglossus, by which these motor fibres, following the abortion of the ganglia, no longer join the ventral roots of the twelfth, but turn forward to join the vagus thereby forming the trunk of the accessory nerve. Since the work of *His*, 88, and *Mall*, 91, on the human embryo, further details in the development of the tenth and eleventh nerves in other mammals have been supplied in the well known papers of *Froriep*, 82, 85, and 01, and by the work of *Robinson*, 92, and *Lewis*, 03. The latter two give us a more accurate description of the so-called ganglionic commissure, than had before existed; although they failed to recognize the full significance of these ganglia and their relation to the precervical ganglia of *Froriep*.

The comparative morphology of the occipital nerves, particularly with regard to their bearing on the segmental origin of the head, has been the subject of much speculation, ever since *Gegenbaur*, 72, published his work on the selachian head. The charge may perhaps be justly made that more space in the literature is given to theories and discussions concerning these structures than to actual observations on their comparative and embryological anatomy. This subject will be briefly treated under the heading comparative morphology. It will be emphasized there that the ganglia of the trunks of the ninth and tenth (gang. petrosum and gang. nodosum) are branchio-meric and largely independent of the

ganglia of the roots. The latter ganglia though not segmental more closely resemble the spinal ganglia; the attempt however to reduce the cranial nerves to a spinal nerve type is deprecated. Some such hypothesis as was long ago suggested by *Balfour*, '76, is much easier of application. This investigator supposed the head and trunk to have become differentiated from each other when there was only a mixed motor and sensory posterior root present and no ventral root, as was then supposed to be the case in the amphioxus. Since then it has been found (*Ransom* and *Thompson*, '86, *Hatscheck*, '92, *Dogiel*, '03) that the amphioxus and cyclostomes have ventral, purely motor, roots. These do not arise from the cord at the same level with, nor do they join, the dorsal roots. The two might be spoken of as ventral and dorsal nerves. If then Balfour's hypothesis were modified to fit with our present knowledge, it could be stated as follows: The head and trunk nerves were differentiated from each other at a time when there existed mixed motor and sensory dorsal roots and pure motor ventral roots. These ventral and dorsal roots did not then arise from the neural tube at corresponding levels, and were independent of each other. In the spinal region of higher vertebrates a modification has occurred, by which the dorsal and ventral roots have become strictly segmentally arranged, and have joined in pairs, each pair forming a common nerve, which is situated median to the myotome. In the head region the nerves have retained the primitive type; the dorsal roots still contain a good proportion of motor fibres, and are situated beneath the epidermis and outside of the myotomes. They are not segmentally arranged and do not join with the ventral roots to form common nerves, but form a system of separate ventral and dorsal (lateral) nerves.

MATERIAL AND METHODS.—The elements of the peripheral nervous system do not reach a degree of differentiation, which is sufficient for reconstruction, until toward the end of the third week. From then changes in the form and relation continue until the third month, when the structures have practically reached the condition found in the adult, and development may be considered as completed. The various stages in their growth were thus found to be covered by the embryos listed in the following table (page 86). Their ages have been determined by use of Mall's rule (*Mall*, '03), *i. e.*, the age in days equals the square root of the product of the length times one hundred.

It was found that the ganglion masses and fibre paths could be satisfactorily identified and traced by means of profile reconstructions. This procedure was made use of with all embryos, with the exception of one at a late stage which was large enough for dissection. The details adopted

in its application consisted in making enlarged drawings of the sections, usually fifty diameters, with a projection apparatus or camera lucida, upon separate sheets of transparent paper. Paraffined wrapping paper is serviceable and inexpensive, better than this, being stronger and more transparent, is the "process" paper, used in Germany as "butter-brot paper" and in this country in packing tobacco. When the drawings were completed, the sheets were piled so that adjacent sections were accurately fitted over each other. A vertical line for reconstruction was then established by marking upon each sheet two lines perpendicular to each other, forming a series of crosses which exactly superimposed throughout the entire pile. The individual sections were then plotted off on mm. paper by fitting the crosses to a chosen perpendicular line, the distance between the sections being determined by the thickness of the sections and the enlargement of the drawings in the usual way. Many of these reconstructions are diagrammatically shown as text figures

TABLE OF EMBRYOS STUDIED.

Length.	Age.	Source.	No.	Reconstruction.
4.0 mm.	20 days.	Hertwig Collection.	137	Profile, one side.
4.3 "	20 "	Mall	148	" both sides.
6.9 "	26 "	His	Br3	" "
7.0 "	26 "	Mall	2	one side.
7.0 "	26 "	Gage	Buxton	" "
10.0 "	31 "	Mall	114	" "
10.2 "	31 "	His	KO	both sides.
10.0 "	31 "	His	D1	one side.
13.8 "	37 "	Hertwig	67	both sides.
14.0 "	37 "	Mall	144	" " , wax plate.
17.5 "	41 "	His	FM	one side.
30.0 "	54 "	Hertwig	161	" "
65.0 "	81 "	Mall	Dissection.

(Figs. 1-12), in which fibre masses are represented by lines and ganglion cell masses by dots. The same enlargement is used in all of these so that the actual increase in size may be readily seen by comparing them.

In order to reproduce the third dimension a clay model was made of a 4.3 mm. embryo, and a wax plate reconstruction of a 14.0 mm. embryo. Drawings of these are reproduced in Plates I and II.

Following the suggestion of Dr. Bardeen much assistance was obtained in studying these structures by making dissections of pig embryos for comparison. It was possible in this way to control the various stages from 8.0 mm. upward. In making such a comparison it was found, however, that the ratio between the length of the pig and the development of its nervous system does not exactly correspond to that of the human. The development of the nervous system of the latter is somewhat more

rapid; for example, a 14.0 mm. human embryo shows a stage found in the 18.0 to 20.0 mm. pig embryo. The preparation of these dissections was facilitated by the use of the following method: The entire embryo is mordanted for several hours in a solution of potassium bichromate 2.5 per cent, and glacial acetic acid 10 per cent, after which it is rinsed in water and brought into 80 per cent alcohol. The embryo, after a few minutes dehydration in absolute alcohol, is then attached with a drop of thick celloidin to an isinglass strip, previously coated with thin celloidin. Mica or isinglass is used because it can be easily cut to any desired size. By smoking it, before the celloidin coat, it is possible to write any desired label on it, and the black serves as a good background for the embryo. The whole is then hardened for a short time 80 per cent alcohol, and it is then ready for dissection. In order to hold the embryo during dissection the isinglass strip on which the embryo is mounted is clamped to a stage which is made by fastening a glass slide with balsam to one of the facets of a cut-glass polyhedral paper-weight. Such a stage is steady and can be placed in any desired plane. With the embryo firmly mounted in this way the dissection is made under alcohol with a binocular microscope.

DESCRIPTION OF EMBRYOS AT DIFFERENT STAGES.

Embryos of About Three Weeks.

- Hertwig Collection, No. 134..... 4.0 mm.
Mall Collection, No. 148..... 4.3 mm.

(See Figs. 1, 2, 3, and Plate I.)

By the twentieth day the structures have become definite enough in outline to permit of reconstruction. At this stage the ganglion crest of the after-brain and spinal cord has divided longitudinally into right and left halves, each of which has migrated ventro-laterally along the neural tube and forms a flattened cellular band extending caudalwards from the auditory vesicle along the lateral border of the tube to its extreme tip.

That part of the crest which corresponds to the spinal cord consists of compactly grouped cells which are so arranged as to present a flattened continuous portion or *dorsal bridge*, the dorsal border of which is rather smooth and sharply outlined, and shows no connection with the neural tube. There are as yet no dorsal nerve roots. Projecting ventralward from this bridge is a series of rounded segmental clumps of cells which form the primitive spinal ganglia. These end diffusely among the developing fibres of the ventral roots. The ventral border of the ganglionic crest is ill-defined in contrast to the dorsal edge of the bridge of the crest.

At this time the fibres of the ventral roots of the spinal nerves are gathered into loose bundles and can be traced a short distance in the

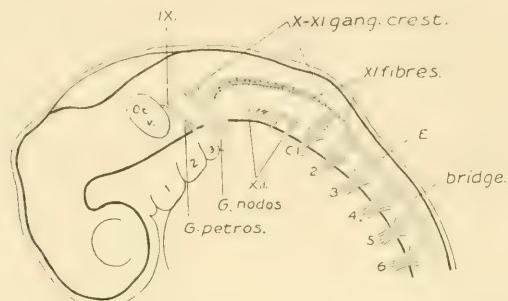


FIG. 1. Reconstruction of peripheral nerves in three weeks human embryo, 4.0 mm. long, Hertwig collection No. 137. Enlarged 16.7 diams.

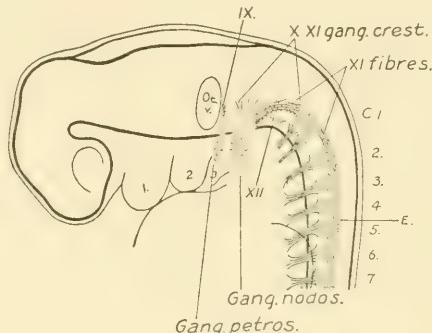


FIG. 2. Reconstruction of peripheral nerves in three weeks human embryo, 4.3 mm. long, Mall collection No. 148. Enlarged 16.7 diams.

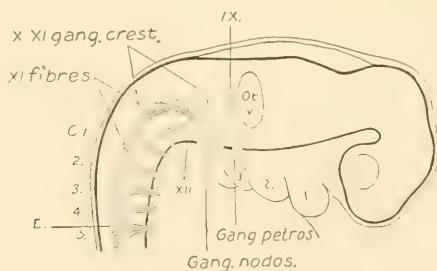


FIG. 3. Reconstruction of right side of same embryo shown in fig. 2.

mesoderm. Among them are many of the so-called sheath cell nuclei. Owing to the fact that the fibres of the ventral roots run through the

ventral part of the spinal ganglia it is impossible to determine the presence or absence of fibroblasts belonging to these ganglia.

The first, or most oral, of the spinal ganglia exhibits special characteristics. It is separated from the second ganglion by an enlarged interval, and it is usually smaller and more slender than the other ganglia. In some cases it consists of a very small clump of cells showing no tendency to unite with its ventral root, or it may be absent completely. On the other hand it may be accompanied by additional ganglionic cell-masses representing the occipital ganglia, found by *Froriep*, 82, to be constant in the sheep. In one case (Fig. 2) the ganglion is divided ventrally into two clumps of equal size, corresponding to a likewise divided ventral root. The more oral of these two may therefore be considered as a persistent occipital nerve and ganglion.

That part of the ganglion crest which is situated lateral to the after-brain differs from the spinal portion of the crest in that it consists of more loosely arranged cells, and as a whole it is flatter and presents an irregularly triangular profile, which at this period does not exhibit a segmental character. That part of it adjacent to the auditory vesicle, and which represents the anlage of the ganglion of the root of the glosso-pharyngeal nerve, is in most cases completely separated from the rest of the crest. It is continued ventralward into the third branchial arch by a looser zone of cells which connects it, just dorsal and caudal to the second gill cleft, with a rounded compact clump of cells forming the future ganglion petrosum. In a similar manner the ganglionic crest of the vagus at its oral end is continuous by means of an ill-defined zone of cells with the anlage of the ganglion nodosum. This ganglion, as can be seen in Figs. 1, 2, 3, and Plate I, is larger than the ganglion petrosum. It is somewhat spindle-shaped, and owing to the branchial arches it takes a caudal direction ending diffusely beyond the fourth arch. Directly over it lies a patch of thickened epidermis and is apparently adherent to it. A careful search was made here and in the ganglion petrosum for evidence of interchange of cells between the ganglia and the epidermis without success. That these ganglia are not simply ventral projections of the ganglion crest is in a degree indicated by the zone of less well-defined tissue, which at this stage separates them from the crest proper.

In the ganglion crest, in addition to the cellular elements, there are a few fibres found at the junction of the crest with the neural tube, thus completing the anlagen of the glosso-pharyngeal and vagus nerves. A greater fibre development is found in the caudal part of the ganglion crest of the vagus, where inclosed among the cells, as in a sleeve, is found a well-defined bundle of fibres representing the accessory nerve. This

bundle makes its appearance at the level of the fourth, fifth, or sixth cervical segment. It consists of fibres which are contributed to it at irregular intervals by the dorso-lateral border of the neural tube. Increasing in size it extends forward, running mesially to the ganglion crest until it reaches the crest of the vagus where the cells of the crest form a sheath for it. The tip of this nerve reaches to the dorsal border of the column of cells which extends from the crest to the anlage of the ganglion nodosum.

The hypoglossal nerve is represented by a row of four or five rootlets, which are in a direct line with those forming the ventral roots of the cervical nerves, and resemble them with the exception that they successively taper off smaller as they become more oral. At this stage they have not grown far enough out for coming together to form a common trunk. The rootlets are bundled together in three divisions, between which are situated the first two occipital myotomes. The third myotome lies between the last division of the hypoglossal and the first cervical nerve.

On looking back at these embryos of the third week, an important feature is observed in the fact that the main motor elements are indicated at this time by the presence of fibres. We find a few fibres in the roots of the ninth and tenth nerves; the trunk of the eleventh is definitely laid down, also the roots of the twelfth and the ventral roots of the spinal nerves. The sensory elements, however, are only indicated by the cellular masses of the ganglion crest.

Embryos of About Four Weeks.

His Collection, Embryo Br3	6.9 mm.
Mall Collection, No. 2.....	7.0 mm.
Gage Collection, Buxton Embryo.....	7.0 mm.

(See Figs. 4, 5 and 6.)

In this group of embryos the ganglionic crest is increased in size, but aside from a disproportionate increase in fibre elements, it presents much the same appearance as seen in embryos of three weeks. The flattened dorsal border of the spinal crest still forms a continuous bridge along the tops of the primitive ganglia. Cropping out along this bridge numerous fibroblasts are seen attaching themselves to the spinal cord. These are the primitive dorsal nerve roots. Attention is directed to the later and slower development of these as compared with the ventral roots. Aside from that seen in the dorsal bridge the ganglia show as yet little or no fibre formation. They consist of cells whose round nuclei, scantily sur-

rounded by protoplasm, are compactly clumped together, the whole forming a row of pillow-like bodies whose ventral borders are lost among the fibres of the ventral roots. It was not possible to determine as to whether or not the ventral borders of the ganglia are involved at this stage in fibroblast formation, and contribute fibres to the nerve root, because of

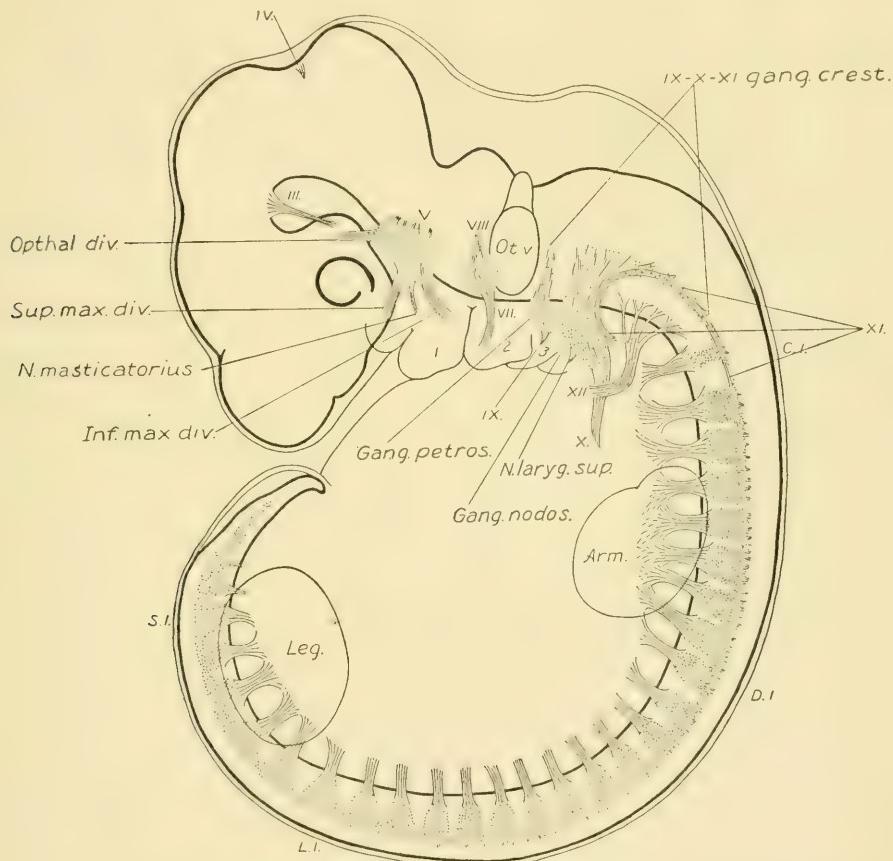


FIG. 4. Reconstruction of peripheral nerves in four weeks human embryo, 6.9 mm. long, His collection Embryo Br3. Enlarged 16.7 diams.

the difficulty of differentiating them from the mesodermal sheath cells of the ventral roots.

The fibres of the spinal nerve roots form compact bundles which branch, as they extend forward, and form intersegmental anastomoses. The profuse anastomosis of the nerve roots from the fourth cervical to the first dorsal segment marks a primitive brachial plexus. (See Fig. 4.)

The roots of the caudal end of the cord are somewhat tardier in their development, and at this stage the lumbar plexus is only slightly indicated.

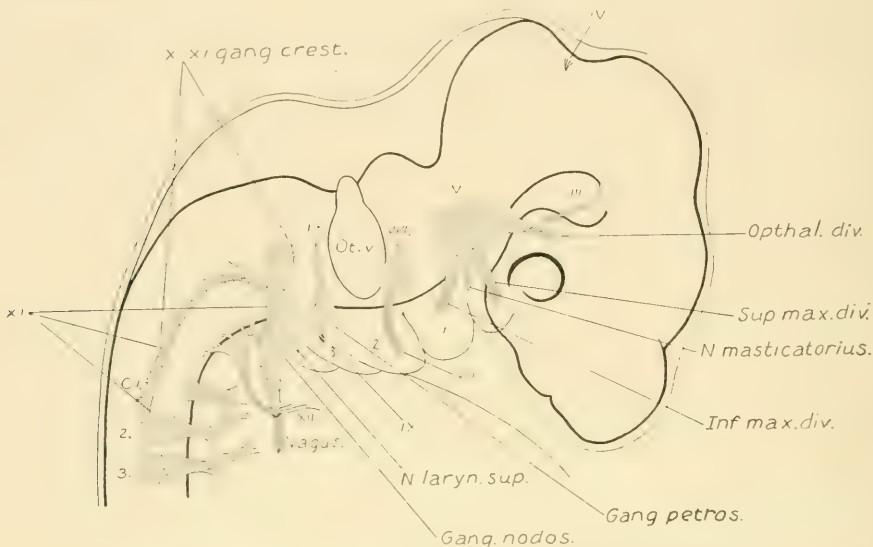


FIG. 5. Reconstruction of right side of same embryo shown in fig. 4.

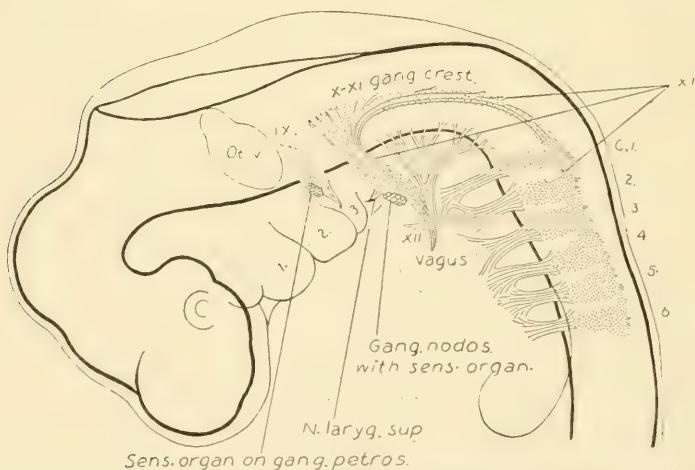


FIG. 6. Reconstruction of peripheral nerves in four weeks human embryo, 7.0 mm. long, Mall collection No. 2. Enlarged 16.7 diams.

On the right side of Embryo Br3 (see Fig. 5), and on the left side of the Buxton Embryo, the first cervical ganglion consists of but a small clump of cells showing no connection with the ventral root, which root,

however, is well developed. Cases of this kind have in the adult the appearance of entire absence of this ganglion, a point which will be taken up later.

The caudal portion of the ganglion crest of the after-brain is longer and more slender than in the previous stage. The accessory nerve is still ensheathed by the cells of the crest. As it extends forward it turns the curve on the back of the trunk of the vagus, and then freeing itself from the vagus it extends a short distance lateralward and ends abruptly in a mass of condensed mesoderm, the anlage of the *m. sterno-cleido-mastoideus*. The oral end of the ganglion crest of the vagus is connected with the ganglion nodosum (it will be observed that this ganglion is not considered as simply a part of the vagus crest, which is because of the apparent independence of the two seen in three week embryos) by a compact mass of cells, among which are found some fibres. From the ganglion nodosum there arise two distinct fibre bundles, ventrally the superior laryngeal, and ventro-caudally the main trunk of the vagus, around which winds the hypoglossal nerve.

The rootlets of the hypoglossal nerve unite and form a stem, as in the adult, which seems to be joined by fibres from the first and second cervical nerves at the point where it bends upward to reach the anlage of the tongue. The descending branch of the hypoglossal can be identified on the right side of Embryo Br3 as a short bud at the point where the nerve crosses the main trunk of the vagus. Thus the descendens hypoglossi develops in this case simultaneously with the appearance of anastomoses between that nerve and the upper cervical nerves.

The root of the glosso-pharyngeal nerve is definitely connected with the ganglion petrosum by a fibro-cellular mass. Ventral to the ganglion the trunk of the nerve is represented by a fibre strand extending into the third branchial arch. In Fig. 6 is represented the area on both the petrosal and nodosal ganglia which still remains attached to the overlying thickened epidermis. In Embryo Br3, on both sides of the embryo, the ganglia nodosum and petrosum appear to fuse.

On review of this group it is seen that at the end of the fourth week the ganglion crest is not yet entirely differentiated. We find laid out, however, the roots, the trunks, and the ganglia of the trunks of the ninth and tenth cranial nerves. Further, all the elements of the eleventh and twelfth nerves are present, and the dorsal and ventral roots and the plexuses of the spinal nerves.

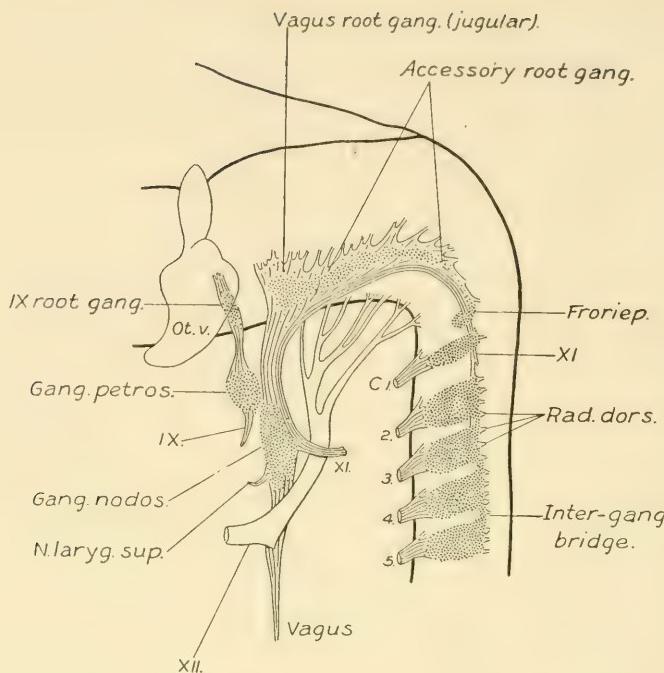


FIG. 7. Reconstruction of peripheral nerves in thirty-one days human embryo, 10.2 mm. long, His collection Embryo KO. Enlarged 16.7 diams.

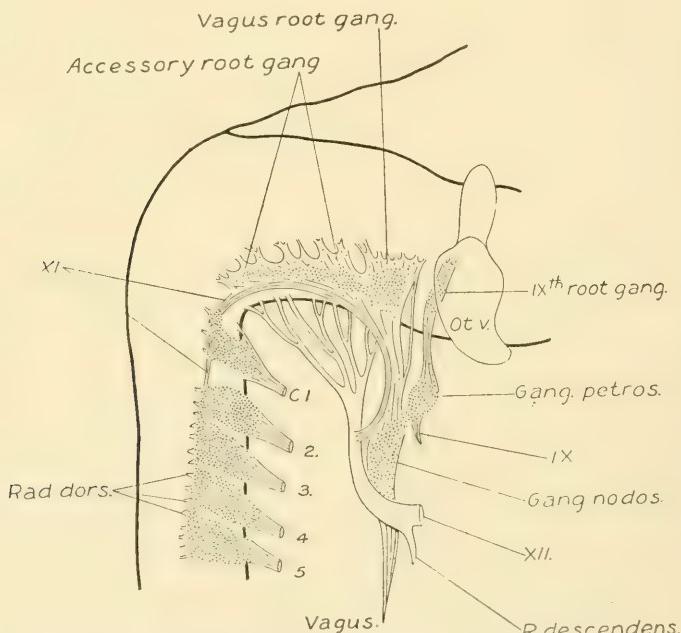


FIG. 8. Reconstruction of right side of same embryo shown in fig. 7.

Embryos of About Thirty Days.

Mall Collection, No. 114..... 10.0 mm.

His Collection, Embryo Dl..... 10.0 mm.

His Collection, Embryo KO..... 10.2 mm.

(See Figs. 7, 8, 9 and 14.)

On coming to embryos 1.0 cm. long the final steps in the transformation of the occipital ganglion crest into cranial nerve rootlets and their ganglia may be seen. When Figs. 7 and 8 are compared with Fig. 4, a considerable increase in the actual mass of cells is observed in this region, and also a marked growth of the fibre elements. As this fibre formation

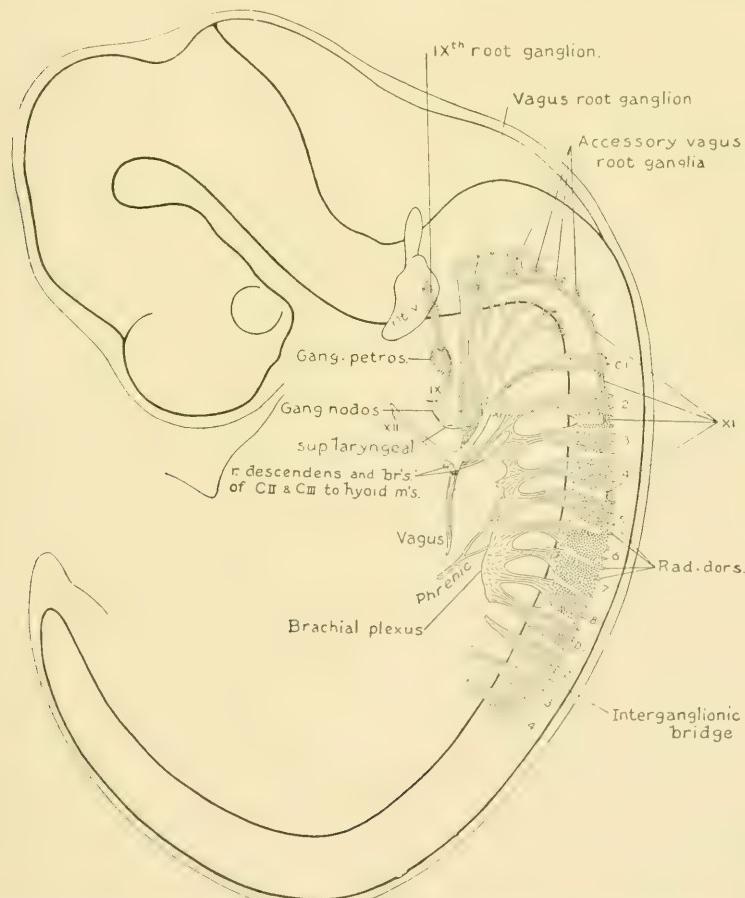


FIG. 9. Reconstruction of peripheral nerves in thirty-one days human embryo, 10.0 mm. long, His collection Embryo Dl. Enlarged 16.7 diams.

continues the cell masses become broken up and separated into ganglionic clumps. Instead of the uniform cellular crest seen in the occipital region in Fig. 4 we find in Fig. 9 a chain of ganglia lying among the rootlets of the ninth, tenth, and eleventh nerves. Just cephalad to the first cervical ganglion, in Fig. 7, is a cell mass which may be regarded, either as a fragment which has become separated off from the first cervical ganglion, or what is more likely a persistent occipital ganglion (precervical ganglion), such as is found by *Froriep*, 82, in the sheep.

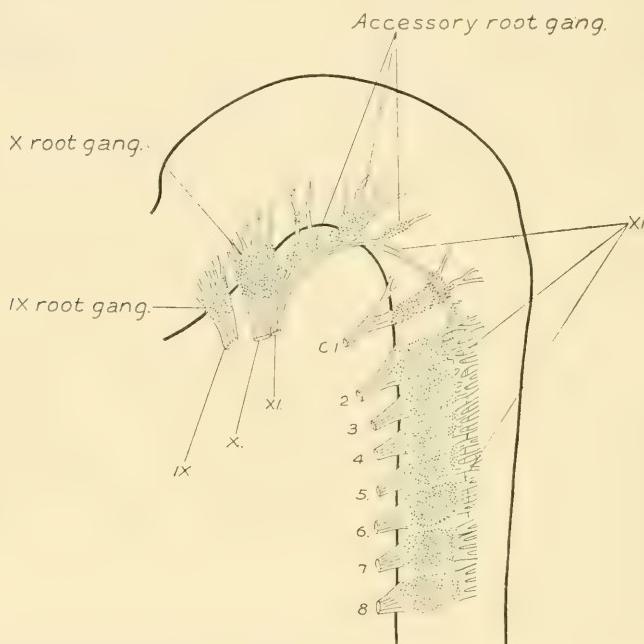


FIG. 10. Reconstruction of peripheral nerves in five weeks human embryo, 13.8 mm. long, Hertwig collection No. 67. Enlarged 16.7 diams.

Embryos of Five to Seven Weeks.

Hertwig Collection, No. 67.....	13.8 mm.
Mall Collection, No. 144.....	14.0 mm.
His Collection, Embryo FM.....	17.5 mm.

(See Figs. 10, 11, 12 and Plate II.)

At the end of the fifth week the ganglion crest is completely resolved into a series of more or less segmental cell masses. The dorsal ridge, which formed an intersegmental bridge across the tops of the spinal ganglia, has disappeared. Simultaneously with the disappearance of this

structure occurs the outgrowth of the central rootlets of the ganglia. On comparing Figs. 1, 9, and 11 one gets the impression of an actual conversion of the dorsal bridge into the ganglion rootlets. In Fig. 10 the dorsal rootlets have attained a considerable length, and show a tendency toward anastomosis.

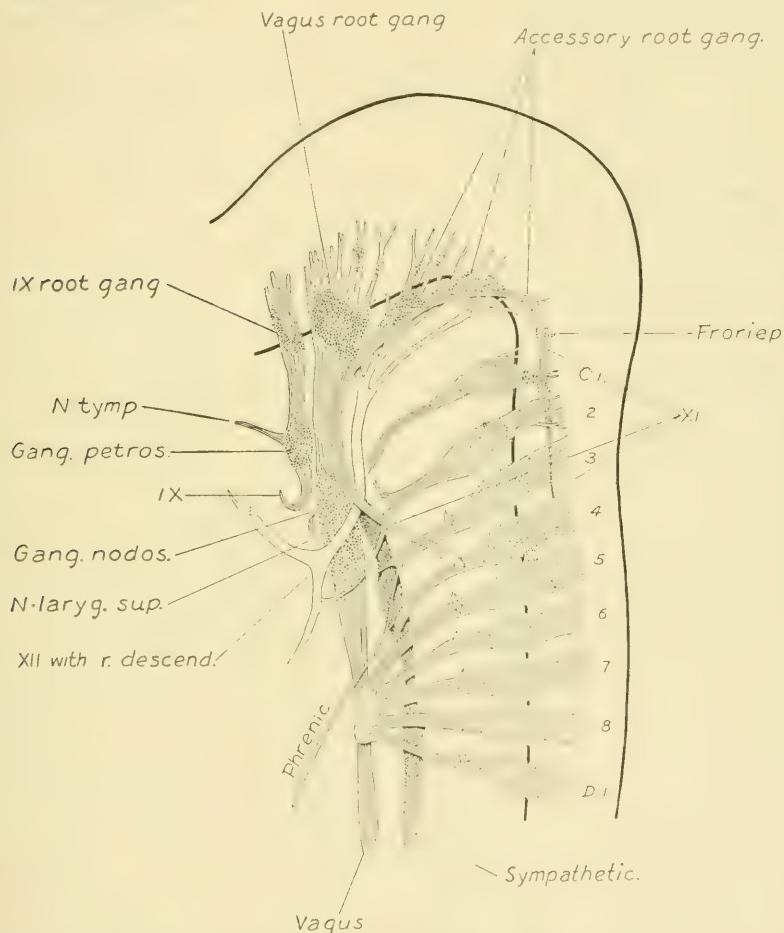


FIG. 11. Reconstruction of peripheral nerves in five weeks human embryo, 14.0 mm. long, Mall collection No. 144. Enlarged 16.7 diams. This drawing is reversed right for left.

The eleventh cranial nerve lies median to the dorsal rootlets, and is attached at irregular intervals to the spinal cord, just ventral to their attachments to the cord. It may run either mesially or laterally to the rootlets of the first spinal ganglion, and is usually adherent to the ganglion itself. Cell masses are found on the trunk of the nerve in this

region, as though fragments of this ganglion. The close relation between the first cervical ganglion and this nerve serves to explain the conditions found in the adult. Along the more cranial portion of the nerve there is a row of ganglia, the *accessory root ganglia*, which become successively larger as we go forward, and which form a series with the ganglion jugulare of the vagus. The number of these accessory ganglia is usually three or four principal masses, and in addition there are several smaller clumps scattered among the rootlets. In a series of pig dissections at the corresponding age it was not possible to determine a true segmental order in their formation, and there was no correspondence between these ganglia and the number of the hypoglossal roots, and they show no connection with them. Thus they are not to be confused with the occipital ganglia of Froriep. The fibre elements of the accessory nerve fuse with those of the vagus. The occurrence of an actual interchange of fibres between them cannot, however, be determined. Leaving the vagus at the ganglion nodosum the accessory can be traced through the *m. sternocleido-mastoideus* to the *m. trapezius*.

The relations of the roots, ganglia, and trunks of the ninth and tenth nerves were seen in the previous stage (Figs. 7, 8, and 9) to have taken on the adult type. In Figs. 11 and 12 the resemblance to the adult conditions is more complete owing to the relative increase of fibre elements. The glosso-pharyngeal nerve arises by several compactly bundled rootlets attached to the neural tube median and caudal to the cartilaginous mass in which the internal ear is embedded. Among these rootlets is the ganglion mass which forms the ganglion of the root or Ehrenritter's ganglion. Beyond this begins the trunk of the nerve, on which is found a second ganglion, the ganglion of the trunk. It is to be remembered that the ganglion of the root and the ganglion of the trunk have developed separately, and have so far remained discrete structures. From the ganglion petrosum is given off ventrally the tympanic branch, or nerve of Jacobson, and caudally the main trunk of the nerve, which hooks inward and forward toward its terminal distribution. The ninth and tenth nerves lie closely together and there is ample opportunity for anastomosis between them; especially between the ganglia of the trunks. It will be recalled that in a younger embryo (Fig. 4) these ganglia were apparently continuous.

The vagus presents the same general type as the glosso-pharyngeus; the root and trunk ganglia are larger, and the trunk itself may be traced down into the thorax.

In Fig. 11 the chain of cervical sympathetic ganglia is indicated, and in Fig. 12 is shown their connections with the spinal nerves. The upper

portion of this ganglionic chain fuses with the ganglion nodosum, and above this it gives off its branches to the carotid plexus.

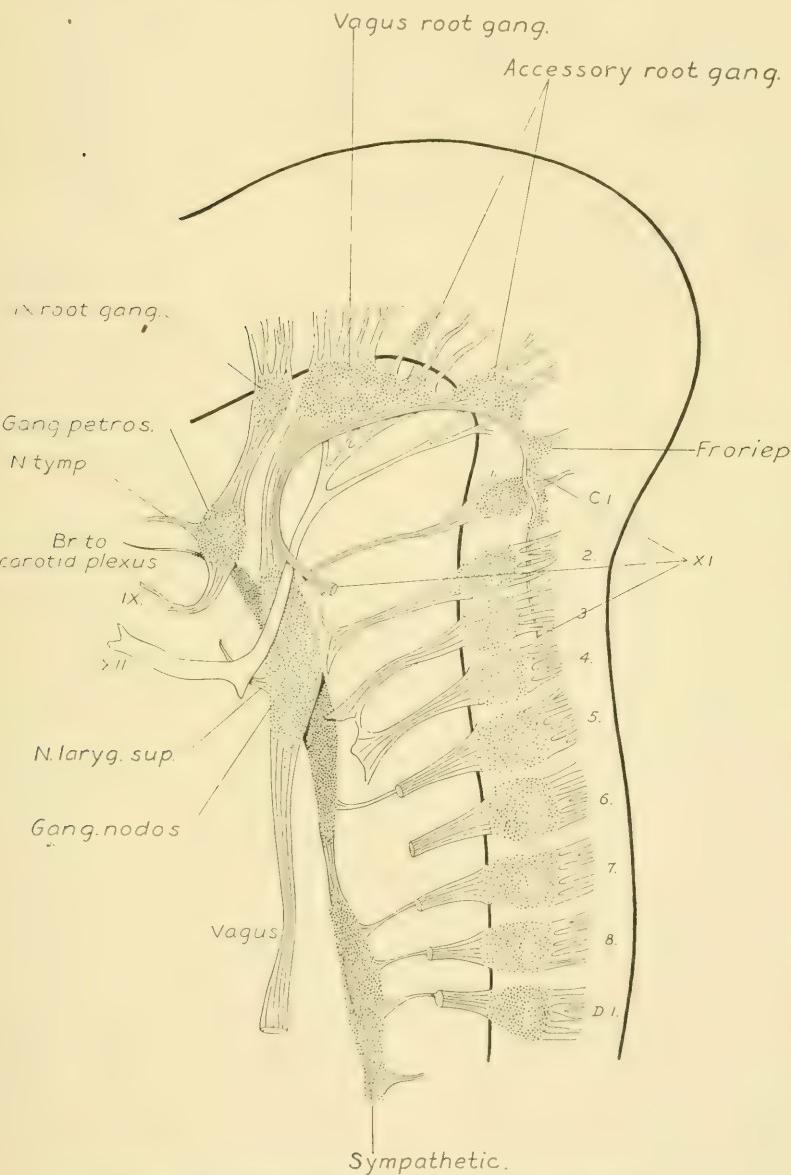


FIG. 12. Reconstruction of peripheral nerves in six weeks human embryo, 17.5 mm. long, His collection Embryo FM. Enlarged 16.7 diams.

The principal branches and communications of the hypoglossal and cervical nerves may be distinctly traced in embryos of this size. It will be seen that we thus have in this group the completion of the principal features of both the fibre and the cellular elements of the nerves under consideration; but the picture presented by the adult structure varies somewhat from this, owing to a disproportionate growth of some parts over others, and instead of what here appears as a ganglion cell predominance we meet there with a predominance of the fibre elements.

Embryos of the Second and Third Month.

Hertwig Collection, No. 161.....	30.0 mm.
Mall Collection.....	65.0 mm.

(See Plate III.)

During the second and third months there is a progressive growth of the fibre elements with a corresponding stretching-out of the nerve trunks and rootlets, which results in a greater separation of the ganglion masses from one another. The further growth of the ganglia is not uniform; while the ganglia of the trunk of the ninth nerve, and of the root and trunk of the tenth, and the spinal ganglia continue in their development, the ninth root ganglion and the root ganglia of the eleventh reach at this time a point of development at which they remain stationary.

In a reconstruction of the left side of the Hertwig embryo, No. 161, which is not here reproduced, the noticeable change from the conditions shown in Fig. 12 is in the length and sharper definition of the trunk of the accessorius nerve. On the trunk of this nerve, between the first cervical ganglion and the ganglion jugulare, there are two root ganglia, and as the accessorius trunk joins the vagus there is a third ganglion mass, which, however, is partly fused with the ganglion jugulare. There are also small clumps of cells among the rootlets of the vagus, as well as on some of the central rootlets of the spinal ganglia.

A dissection of this region in an embryo at the end of the third month is shown in Plate III. There the ganglia of the root of the ninth and the eleventh nerves present very little enlargement. They can be distinguished from the fibre bundles only by a greater opacity, and appear as white nodes in the roots of the respective nerves. The first cervical ganglion is well developed. An arrest in the growth of this ganglion, similar to that in the ganglia just mentioned, might also be expected.

As the nerve fibre growth continues, the last trace of these rudimentary ganglia is lost to the naked eye. In order to determine their ultimate fate a series of sections was made through the structures of this region in an

adult specimen. This shows the presence of persistent clumps of normal appearing ganglion cells, situated along the trunk of the eleventh and on the roots of the ninth and tenth nerves. A diagrammatic reconstruction of the series is shown in Fig. 13. In this case the first cervical nerve received a communicating branch from the accessory, but macroscopically no ganglion was present. In the series, however, this ganglion is represented by a circumscribed group of cells on the trunk of the accessory. Among the rootlets in the same region are scattered groups of cells which may have been separated off from it. On going further forward other small ganglion groups are met with, either just beneath the connective tissue sheath of the nerve roots, or among their fibres, and usually near

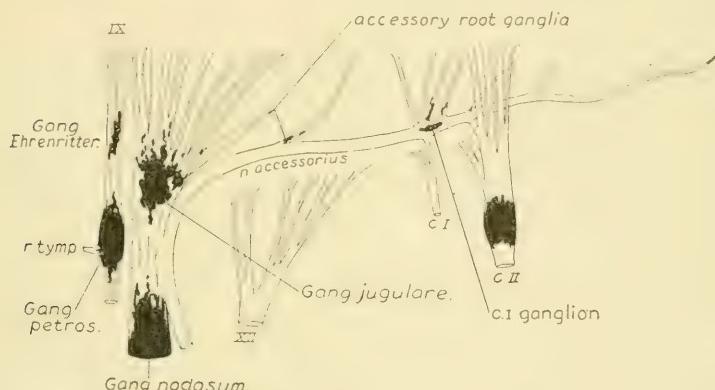


FIG. 13. Diagrammatic reconstruction of ganglion cell masses in peripheral nerves of occipital region in human adult. Compare with Plate IV.

the junction of the roots with the larger trunks. From their position these are considered to be the persistent accessory root ganglia. Although rudimentary in size they are made up of cells which have all the appearances of functioning ganglion cells. It is possible that it is these that provide the sensory fibres for that branch of the accessory which joins with fibres from the vagus to form the pharyngeal branch, in some such way as is schematized in Plate IV.

DEVELOPMENT OF INDIVIDUAL NERVES.

It has been seen, in tracing the development of the ganglionic crest of the after-brain, that the ninth nerve stands apart from the more caudal nerves and develops independently, and apparently uninfluenced by them. The tenth and eleventh, in contrast, are parts of a single complex, and cannot be taken up as individual structures without adopting a sepa-

ration that would be artificial; they will therefore be described together. The hypoglossus and the cervical nerves, where a close relation also exists, will be likewise treated.

The **Glosso-Pharyngeal Nerve** from the beginning possesses the characteristics of a mixed nerve. In embryos 4.0 mm. long (Fig. 1) it consists of a small clump of ganglionic cells, which can be distinguished in the mesenchyma attached to the neural tube just caudal to the otic vesicle and extending toward the third branchial arch. This group of cells represents the anlage of the ganglion of the root, or Ehrenritter's ganglion. Among these cells are a few fibroblastic processes, which do not belong to them, but arise from cells of the neural tube in the dorsal part of the ventral zone of His, and form the motor elements of the root. The character of this anlage resembles that of the vagus; and may be regarded as a part of the ganglion crest of the after-brain, though it is not continuous with the vagal portion of it. It is evident that this ganglion is not a part which has become separated off from the ganglion petrosum as described by Henle, and others (see *Thane*, 95), but is an independent structure. Its inconstancy is to be explained by its further development. It reaches a size early in the embryo at which it ceases to further develop, in some embryos earlier than others. The fibre elements, however, continue to grow, and finally overgrow the ganglion and thus cause it to be apparently absent. A similar occurrence will be seen in case of the root ganglia of the accessory nerve.

Ventral to this group of cells is a somewhat larger clump of cells, the primitive ganglion petrosum, which is situated directly beneath the epidermis at the caudal and dorsal margin of the second gill cleft (see Figs. 1, 2, and 3). At this time it is separated from the rest of the anlage of the ninth by a looser zone of cells, and this gives it the appearance of having developed *in situ*, rather than of being a subdivision or bud from the rest of the anlage. It is true that the same appearance might arise from a migration of cells from the latter followed by a proliferation of them at this point. The position of the ganglion petrosum here and in older embryos (Figs. 4, 5, and 6) indicates a close relationship between it and the branchial arches, and the same is likewise true of the ganglion nodosum. In this respect the anlagen of the ganglia of the trunks differ from the ganglionic crest proper, or anlage of the root ganglia, which is well removed from the branchial arches and does not show any trace of branchio-meric arrangement. This suggests a difference in origin to exist for the two kinds of ganglia. Another point of difference between the ganglia of the roots and the ganglia of the trunks of these nerves is the

fusion of the latter with an overlying patch of thickened epidermis, and an apparent absence of such a fusion in case of the ganglia of the roots.

The relation existing between the ganglia of the seventh, ninth, and tenth nerves and the overlying epidermis has been described in mammals by *Froriep*, 85, and it is regarded by him as the anlage of rudiments of the phylogenetically lost branchial sense organs of *Beard*, 85, and *van Wijhe*, 82. In elasmobranchs *Froriep*, 91, describes later a double line of fusion between ganglia and epidermis, forming the lateral and epibranchial sense organs, which may perhaps be considered as comparable to the ganglia of the roots and ganglia of the trunks. In mammals, however, he had found only a single line of epidermal fusion, that existing over the ganglia of the trunks. In our series of embryos the ganglia of the roots do not seem to take part in the formation of epidermal sense organs, and show no sign of fusion. The condition here resembles that described by *Froriep*, 85, in his earlier paper. The adherence between the epidermis and the ganglion petrosum and nodosum is indicated in Fig. 6. It is found in all the human embryos studied from 4.5 to 7.0 mm., after which it disappears. This is a somewhat earlier and briefer period than given by *Froriep* for other mammals. No indication of interchange of cells between ganglia and epidermis could be made out.

The ganglion petrosum in embryos 7.0 mm. long has become connected with the ganglion of the root by a definite strand of mixed fibres and cells, the fibre elements more and more predominating as the embryo becomes older. At the same time a tapering bundle of fibres sprouts from the distal end of the ganglion petrosum, and forms the main trunk of the nerve, the ramus lingualis, and supplies the third arch. Another branch appears in 14.0 mm. embryos, the ramus tympanicus, and extends forward into the second arch. The ganglion thus gives off a branch both oral and caudal to the second gill cleft, and this completes the glossopharyngeus as a typical visceral arch nerve. This was pointed out in mammals by *Froriep*, 85, who regards the r. lingualis as the post-trematic and the r. tympanicus as the pre-trematic branch.

Communications exist between the ganglion petrosum and the ganglion nodosum in an embryo of 7.0 mm. (Figs. 4 and 5), where they seem almost as a continuous structure; in other embryos of this stage, and younger, they are completely separated. Later (Figs. 7, 8, and 9), following the relative change in position of the adjacent parts which succeeds their unequal growth, these structures are gradually brought close together, and secondary communications are established between them.

The **Vagus Complex** includes both the vagus and accessory divisions, the tenth and eleventh cranial nerves, which develop practically as a single structure; though the complex is more spread out than the trigeminal, yet the relation of the accessory to the vagus is embryologically much the counterpart of that of the motor root of the trigeminus to the rest of that nerve. To speak of the two divisions as individual cranial nerves is misleading; perhaps a new terminology should be introduced, which would express more exactly their comparative and embryological relations. *Onodi*, 02, has suggested the entire removal of the name *accessorius* as an independent cranial nerve, but does not himself attempt to carry it out. Eventually such a radical attack upon the nomenclature may prove advisable; in this paper, however, whenever it is necessary to distinguish between the different parts of the vagus complex, the original usage will be retained which is based on the gross anatomy of adult specimens: the term "vagus nerve" will be applied to that portion of the complex represented by the ganglion jugulare with its rootlets, and the peripheral nerve trunk extending from this on which is found the ganglion nodosum; the term "accessory or eleventh cranial nerve," no distinction being made between vagal and spinal portions, will refer to the remainder of the complex situated caudal to this, and includes ganglionated rootlets and the large motor trunk extending peripherally to the sterno-cleido-mastoid and trapezius muscles. In their development it will be seen that both divisions contain motor elements, which spring in a continuous line from the lateral border of the neural tube as far down as the third or fourth cervical segment, and sensory elements which are developed from the cells of the ganglion crest. Later, following its further growth, the oral or vagus division of the complex becomes predominantly sensory, and the caudal or accessory division predominantly motor.

The ganglion crest of the after-brain is apparently directly continuous with that of the spinal cord, and extends from the first or second cervical ganglion to the otic vesicle, an interruption indicating the division between the ninth and tenth nerves. We agree with *Dohrn*, 01, who describes the vagus crest as forming a unit with the spinal crest, rather than with *Froriep*, 01, who distinguishes between a ganglion crest of the head and one of the trunk, and states that they do not simply go over into one another, but overlap and run along adjacent to each other, each ending for itself. Evidence of such an overlapping could not be made out.

An embryo of 4.0 mm. represents the youngest stage at which the crest was sufficiently differentiated from the mesoderm for accurate recon-

struction. The shape of the crest at this time is represented in Figs. 1, 2, 3, and Plate I. In the figures the presence of developing nerve fibres are diagrammatically shown among the cells of the crest. Small bundles of these fibres spring at irregular intervals from the lateral angle of the neural tube and enter the crest. In the caudal two-thirds these fibres join to form a definite strand, which is the primitive trunk of the accessory nerve. This trunk reaches down into the region of the spinal ganglion crest to the level of the third or fourth cervical segment. The fibres do not, however, enter this crest, but run along median to it as far as the first cervical, when they enter the vagus crest as though into a sleeve. In regard to the first cervical a variability is shown, the trunk may run median, lateral, or through it. Forward, in a line with these fibres of the accessory trunk, are found a few others forming small scattered bundles at the head of the crest. That the fibres which are present at this stage are motor may be inferred from three facts: firstly, they spring from the lateral horn region of the neural tube; secondly, there is at this time no apparent fibroblast development in the cells of the ganglion crest; and thirdly, some of them can be followed in their further development until they become known motor elements, as in the case of the main trunk of the accessorius, and as in the spinal cord where the ventral roots at this time are well laid out, though there is as yet no trace of dorsal roots.

Ventral to the head of the vagus crest, and partially separated from it by a looser zone of cells, is found a second ganglionic mass, the primitive ganglion nodosum, the relation between which and the crest repeats the condition found between the ganglion of the root and the ganglion of the trunk of the glosso-pharyngeus, evidence of independence being here equally strong. As was there pointed out, the ganglion nodosum is closely associated with the development of the more caudal branchial arches. The patch of thickened epidermis over the ganglion, as in Fig. 6, represents an epibranchial sense organ. A complete segmentation of the ganglion nodosum, which might be expected in considering this anlage as the morphological equivalent of a series of gill cleft ganglia, is not found, though the cells show at first a loose irregular grouping, which represents perhaps a branchio-meric tendency. The laryngeal branch of this ganglion is present in 7.0 mm. embryos, and forms the principal nerve to the fourth branchial arch. The main vagus trunk is differentiated at about the same time and is seen sprouting out from the distal end of the ganglion.

At the end of the first month (Figs. 7, 8, and 9) the cellular column between the ganglion nodosum and the ganglion crest is converted into a fibrous trunk. At this time the ganglion crest, besides an increase in

size, is modified in form by the development of numerous rootlets attaching it to the neural tube, and by an irregular clumping together of the cells of the crest, forming ganglion masses along the main trunk of the accessorius, which now lies at the ventral border of the crest. The rootlets which are developed at this time are in part sensory, as is evidenced by comparison with the dorsal spinal roots. The division of the crest into ganglion masses is accompanied by a rapid development of fibres between its cells, and it is probable that the growth of these fibres is the cause which spreads the cell masses apart into separate clumps. Such a separation into clumps radically differs from true segmentation, the latter does not seem to occur here. As the fibre growth continues the ganglion masses become more and more separated, and finally the crest becomes completely converted into a series of discrete ganglia (Figs. 10 and 11). The most oral one is the largest and forms the vagus root ganglion, the ganglion jugulare. Caudal to this, successively diminishing in size, is a chain of three or four accessory root ganglia, which extend backward along the accessory trunk until they meet the cervical ganglion series. In the vagus complex the ganglia diminish in size in the caudal direction, while in the spinal series the reduction in size is in the oral direction; this fact enables one to distinguish between accessorius root ganglia and precervical (Froriep) ganglia. Those ganglion masses found adherent to the accessorius between the first and second, and the second and third cervical ganglia, as in Figs. 10, 11, and 12, are developed from the spinal crest, and represent nodules derived from the spinal ganglia and which have become separated off. Formed similarly to these, are found isolated masses on the rootlets of the jugular ganglion.

The *vagus division* of the complex at this stage (embryos of 14.0 mm.) therefore consists of mixed motor and sensory rootlets, the ganglion jugulare, and the nerve trunk on which is situated the ganglion nodosum, giving off a laryngeal branch as well as communicating branches to the ganglion petrosum. The *accessory division* begins at the third or fourth cervical segment. Its trunk runs median to the dorsal roots, except at the first cervical where it may be lateral. It is attached to the neural tube by mixed rootlets; on which are found a varying number of ganglia. The trunk after an arched course joins the vagus division, but the greater portion of it soon leaves the vagus and extends to the shoulder region and supplies the sterno-cleido-mastoid and trapezius muscles.

The essential features of embryos of the fifth and six week, Figs. 11 and 12, will be observed to be the same as in older embryos and in the adult, Plate III and Fig. 13. The existing difference may be accounted

for by the disproportionate growth of the fibre elements over that of the cellular elements, and of some of the cellular masses over that of others; most of the cell masses persist, but some of them early reach a point at which they remain stationary, such as the accessorius root ganglia, and sometimes the first cervical. Following the increase in fibre growth they become buried among the rootlets or on the accessorius trunk, and though not seen by the naked eye they can be seen on section. Fig. 13 represents a case in which the first cervical ganglion was macroscopically absent, but microscopically it is present as a large clump of normal appearing ganglion cells within the sheath of the accessorius trunk.

Anastomoses between the first cervical and the trunk of the accessory nerve in the adult have excited much interest. Among others they have been studied by *Kazzander*, 91, and later by *Weigner*, 01. A study of Weigner's drawings shows that the accessory nerve of one side has no constant relation to the accessory nerve of the other side in the same individual; they bear themselves as independent structures, and his 37 examinations may therefore be considered as 74 individual cases. By re-analyzing Weigner's cases in this manner instructive data on our present subject have been obtained. They show that the relation in the adult of the first cervical to the accessorius is as follows:

19%—First cervical ganglion and dorsal root are present, and do not anastomose with the accessorius.

19%—First cervical ganglion and dorsal root are macroscopically absent.

62%—Various kinds of anastomosis between the first cervical dorsal root and the accessorius. In many of these cases the ganglion is macroscopically absent.

These anastomoses are doubtless to be explained on embryological grounds. The relative position of the two structures at the beginning of connective tissue formation would determine their permanent relations. If they lie in contact at that time they become permanently adherent. Secondarily, when the dorsal roots become thus entangled in the accessory trunk, as they are apt to in case of the first cervical, they are dragged along out of their original position by later growth and the consequent relative shifting of all of the structures in that region. Further irregularities in their course may be caused by the accessory which, being laid down earlier than they, would have the tendency to guide the impinging dorsal rootlets out of the direct centripetal line to the neural tube, and along its own trunk, either forward or backward. A diagram showing some of these variations is reproduced in Plate IV. In the same diagram is shown the hypothetical course of some of the other fibres of the acces-

sory. No motor fibres are represented as running from the accessory to the larynx, the absence of such fibres having been well established by the work of *Onodi*, 02, and others, and the clinical observations of *Seiffer*, 03. Fibres of the accessory doubtless join the trunk of the vagus, but they are omitted here for sake of simplicity.

The Hypoglossal Nerve can first be made out in embryos at the end of the third week, at which time it consists of loose fibre strands which can be traced between the occipital myotomes springing from the ground plate of the neural tube and extending a short distance in the mesenchyma (see Fig. 1). These rootlets are formed in three or four segmental groups and develop in the same line with the ventral roots of the cervical nerves. During the fourth week they grow forward and fuse in a common trunk. At the end of the first month this trunk has passed around the ganglion nodosum, and curves around the sinus cervicalis mesially and orally to reach the anlage of the floor of the mouth. A week later its principal branches of distribution are indicated.

As the hypoglossus crosses the ganglion nodosum it gives off the ramus descendens, which is first definitely seen in embryos 1.0 cm. long. *Mall*, 91, and *Piper*, 00, report its absence in embryos 7.0 mm. and 6.8 mm., respectively. *His*, 88, pictures a long r. descendens in Br3 (6.9 mm.). In a reconstruction of the same embryo, made since then by the author (see Fig. 4), this is not seen. There is, however, on the opposite side (Fig. 5) a slight indication of a beginning branch. At the time the descendens is developed the opportunity for communication between the hypoglossus and the upper two or three cervical nerves already exists; that is to say, the terminal fibres of the latter end in brush-like tufts in close contact with the former. The amount of interchange of fibres cannot be accurately traced, but it is evident that the character of the descendens is dependent on the nature of the contribution of fibres from the cervical nerves. The course in the development of this cervical anastomosis is as follows (compare Figs. 3, 4, 6, 9, and 11): The fibres of the hypoglossal and the upper cervical nerves start out perpendicularly from the neural tube, and due to the curve of the latter they come together like spokes in a wheel, and then grow along adjacent to each other into the premuscle tissue of Froriep's schulterzungenstrang; when the formation of the nerve sheaths begins, adjoining fibres become thereby more or less bound together, and as the individual tongue and hyoid muscles draw apart these nerves are led out into an open plexus, the adult arrangement of which and its variations has been described by *Holl*, 77. The exact formation of this anastomosis must depend on the position of the fibres at the time the sheaths are formed. This introduces a variability which

might account for the different arrangements found by Holl. A further source of variation is presented by slight differences in the division line between the rootlets of the hypoglossus and the first cervical; the fibres destined for the r. descendens, for instance, may be either picked up with the more caudal rootlets of the hypoglossal, when there will be little or no communication between the hypoglossal and the first cervical, or on the other hand may be picked up with the first cervical and reach their destination through anastomosis with the hypoglossal. Thus in embryo No. 144 of the Mall collection (Plate II) on the right side the first cervical contributes no fibres to the hypoglossal and descendens, while on the left side a large communicating bundle exists between them.

In the early stages the rootlets of the hypoglossal present a close similarity to the ventral roots of the spinal nerves, and now are generally considered as a cranial continuation of them; the nerve being thus derived from the fusion of three or four segmental spinal nerves, which in the course of phylogenesis have become enclosed in the cranium. In the hypothetical ancestor the segments of the nerve belonged to the trunk, and possessed, in addition to the ventral roots, both dorsal roots and ganglia, the latter becoming subsequently reduced coincidently with the invasion of the vagus group into this region. Strong support to this view was given by *Froriep*, 82, who in the hooved animals found persistent dorsal roots and ganglia belonging to one or two of the more caudal divisions of the nerve. Similar precervical ganglion masses and rootlets were found in the rabbit, cat, and mouse by *Martin*, 91, and *Robinson*, 92. The former describes five hypoglossal ganglia in cat embryos, of which he finds only the most caudal one to persist. He thus apparently includes those that in our series of reconstructions are considered as accessory root ganglia, which we think have a different phylogenetic significance. In the human embryo *His*, 88, describes an abortive precervical ganglion, and names it after Froriep. Inasmuch as he considers the hypoglossus to belong phylogenetically to the vagus rather than to the spinal nerves, he is inclined to doubt a relation between the Froriep ganglion and the hypoglossus. In our reconstructions a typical ganglion may be seen in Figs. 7 and 11. The former is the same embryo pictured by His, and does not essentially differ. On the other side of this embryo, Fig. 8, the first cervical ganglion creeps forward a short distance along the accessorius tract, and thus represents what may be styled as a precervical tendency. An interesting case is shown in Fig. 2, where the first cervical ganglion is divided in two equal parts, each having its own ventral root. With further growth they would have become separated, as the spinal ganglia do, and then we should have in the more oral one

a typical Froriep ganglion with a ventral root that would have doubtless joined with the hypoglossus fibres. In Fig. 9 a slight indication of a ganglion is present, though it is not labelled in the diagram. In such cases one cannot say whether it belongs to the spinal group or to the accessorius root ganglia of the cranial group. These two seem to develop from the same crest, and it could be expected that the oral tendency of the former and the caudal tendency of the latter might cause in some cases a fusing of the two; such an instance is seen in Fig. 12. Where the retrogression of the spinal elements is advanced, the Froriep ganglion is absent, and the first cervical also then shows abortive tendencies. If Fig. 5 is compared with Fig. 7, it will be seen that there the spinal reduction extends an entire segment further caudad; instead of a rootless Froriep ganglion, as in Fig. 7, there is in Fig. 5 a rootless first cervical ganglion.

It is evident that there is a great irregularity in the degree of reduction of the occipito-spinal dorsal roots and ganglia in different individuals. By comparing individuals of different ages we cannot therefore estimate the retrogression undergone in the development of a single individual; one cannot say, for instance, that because a Froriep ganglion is present in an embryo of 7.0 mm. and is not present in another embryo of 14.0 mm. that it has in the latter case disappeared. It was found in case of the accessory root ganglia that ganglion masses once present persist throughout life, though they may early reach a point beyond which they do not further develop. The same is doubtless true as regards the Froriep ganglion.

COMPARATIVE MORPHOLOGY.

In considering the phylogenetic significance of the nerves of the occipital region it becomes apparent that we are here dealing with structures of two different sources; on the one hand, the cranial nerve elements represented by the glosso-pharyngeus, the vagus, and the accessorius—a portion of the vagus, and on the other hand the elements of spinal origin, the upper cervical nerves and the hypoglossus. The literature concerning the comparative anatomy of these structures is voluminous, and particularly their involvement in the various theories proposing a segmental origin of the vertebrate skull. A complete review of this literature and discussion of the morphological bearing of the cranial nerves is given by *His*, 87, and again later by *Rabl*, 92. Since then has appeared the important work of *Fürbringer*, 97, supplemented by the embryological investigations of *Braus*, 99, and *Froriep*, 02. Mention should also be made of the work done on the accessory nerve by *Lubosch*, 99. The general facts as known may be stated as follows:

In the lower fishes the cranial and spinal elements are clearly separated and their territories do not overlap; a line may be drawn oral to which all the nerves are cranial and caudal to which all are spinal. In the phylogeny, owing to a caudal encroachment of the skull into the spinal region, the more oral of the spinal nerves become included in the head region and have special foramina of exit. Those that are thus assimilated by the selachii have been styled by Fürbringer as occipital nerves, and those assimilated in addition later by the holocephali are called occipito-spinal nerves. With this assimilation, however, the spinal and cranial elements are still discretely separated by a transverse line of demarcation. There is no actual overlapping of the two until we come to the sauropsida. Here and in all higher vertebrates, accompanying the conversion of certain vagus-gill muscles into the trapezius and sternocleido-mastoideus, the cranial elements (*i. e.* vagus complex) make a caudal invasion into the spinal region, in such a manner that the accessory portion of the vagus is found wedging itself in between the ventral and dorsal spinal roots mesial to the ganglia, gaining attachments to the cord just ventral to those of the dorsal spinal roots.

In the human embryo the different stages of this invasion cannot be demonstrated. Either the early steps are not repeated in the embryological history of higher types, or it may be, as *McMurrich*, '03, suggests, that the derivation of such structures cannot be demonstrated ontogenetically because the phylogenetic stages occur while the structures are still in an undifferentiated state. In the embryos studied, as soon as the nerve elements can be distinguished, they have their final relative position, and the accessorius is found extending well down into the cervical region. Its caudal end is indicated by "E" in Figs. 1, 2, and 3. The vagus-accessory anlage is, in all three instances, about of the same size. Some variation exists in the extent of overlapping of the cranial and spinal parts, as is evidenced by the variation in distance between the ganglion jugulare and the first cervical ganglion. It is doubtless a variation of the individual, and is of the same character as the variation occurring in the distance over which the accessory nerve extends into the cervical region of the adult. In Fig. 14 is shown the wedge-like invasion of the cranial nerve elements into the spinal territory. The figure is a diagrammatic profile reconstruction of an embryo one month old. The gill arches, vertebral skeleton and muscular apparatus, and spinal and cranial nervous systems are plotted out with view to a comparison of their relative positions. It shows clearly the impossibility of drawing any transverse line through the body, oral to which everything would be cranial, and caudal to which everything would belong to the spinal

system. The behavior of the nervous system adds to the irregularity in the line of junction between the head and trunk.

As the accessorius wedges itself into the spinal territory there occurs a progressive retrogression of the more oral spinal elements, resulting in the disappearance of the dorsal roots and ganglia of the occipito-spinal nerves, these being the first nerves encountered. The ventral roots of these nerves persist and join to form the hypoglossus, and supply the

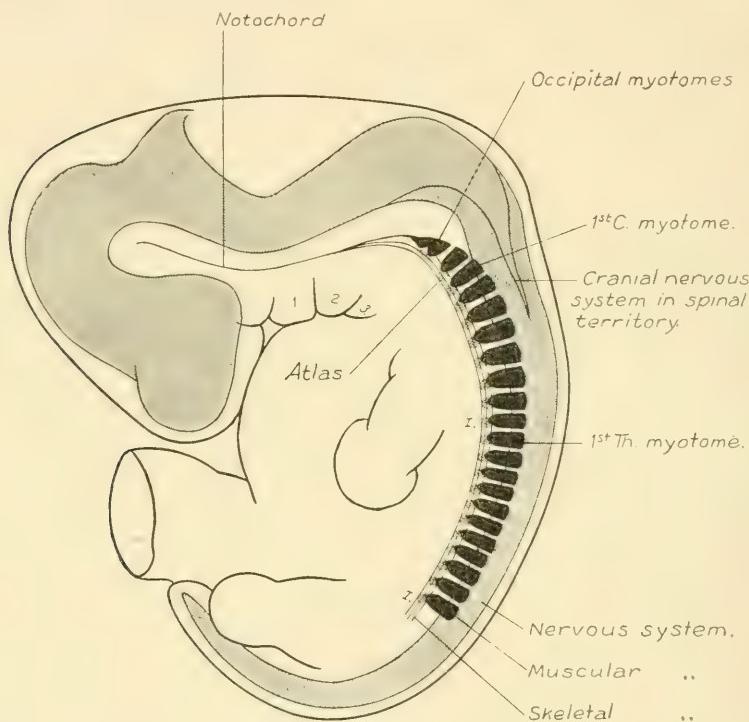


FIG. 14. Diagrammatic reconstruction of one month human embryo, 10.0 mm. long, Mall collection No. 144. Enlarged 9 diams.

tongue, which in the meantime has been acquired in the floor of the mouth. In some of the domestic animals (rabbit, pig, cow, and sheep) one or two of the more caudal of the occipito-spinal dorsal roots and ganglia persist as was pointed out by *Froriep*, 82, whose name they have received. In man the most caudal ganglion occasionally persists, but it is usually without any connection with a corresponding ventral root; in one case, however, a *Froriep* ganglion with ventral root was present, and doubtless would have joined with the hypoglossus as its most caudal root.

Often the connection between the ventral root of the first cervical and its ganglion is also missing in man, and the ganglion rudimentary and found only on section. These rudimentary ganglia during embryonic life become adherent to the invading cranial member, the accessory nerve, and though all connection with the ventral root is absent, they still may functionate by sending their fibres forward or backward along the accessorius, in the latter case joining a more caudal nerve. Although the first cervical ganglion, and perhaps a precervical or Froriep's ganglion may thus lie in the tract of the accessorius, it is to be remembered that embryologically they are separate structures, the one cranial and the other spinal. The apparent relation between the two is only due to the fact that in the early stages they lie closely together, and become adherent in this position.

In addition to these occipito-spinal (precervical, hypoglossal, or Froriep's) ganglia, there are found in the human adult other rudimentary ganglia situated along the accessory nerve, which are of cranial origin, and similar to the root ganglion of the vagus. These are the accessory root ganglia; they form, in the six weeks embryo, a series of ganglionic clumps, which extend caudalward from the ganglion jugulare, successively diminishing in size, along the tract of the accessory nerve attached to its rootlets. A true segmental arrangement of them does not seem to prevail in the human embryo, and the same is true in dissections of pig embryos. The ganglion jugulare continues to develop, but these accessory ganglia early reach a size beyond which they do not further develop. They, however, do not undergo retrograde metamorphosis, at any rate not completely, for evidence of them may still be found in the human adult.

The root ganglia of the ninth, tenth, and eleventh nerves develop from a ganglion crest which has an appearance and history analogous to that from which the spinal ganglia develop. The ganglia which form on the trunks of the vagus and glosso-pharyngeus apparently develop independently from that crest, and they differ from the ganglia of the roots in being branchio-meric, and in possessing definite traces of rudimentary sense organs.

The hypoglossus in contrast to the tenth and eleventh nerves, which show no trace either in rootlets or ganglia that they were ever formed from a series of segmental nerves, presents a distinct segmental grouping of its fibres, as may be seen in Plate I and Fig. 1. This fact, added to its resemblance in its early stages to the ventral roots of the cervical nerves in point of origin from the same column of cells, its relation to the myotomes, and the occasional presence of a Froriep ganglion, offer conclusive evidence that this nerve is the equivalent of three or four ventral

roots of phylogenetically lost occipito-spinal nerves, which have become fused into a single trunk.

CONCLUSIONS.

1. The tenth and eleventh cranial nerves are parts of the same complex, both possessing mixed motor and sensory rootlets, together with root ganglia derived from the same ganglionic crest.

2. During the progress of development of this vago-accessory complex the cephalic end becomes predominantly sensory, and the caudal end becomes predominantly motor and also more spread out. This produces a difference in the appearance of the two portions which has resulted in their being considered as two independent structures. The cephalic portion forms the vagus or tenth cranial nerve, and the caudal portion the n. accessorius Willisii or eleventh cranial nerve. The old nomenclature is retained, and in so doing the term eleventh cranial nerve is used as synonymous with n. accessorius vagi plus n. accessorius spinalis.

3. The root ganglia of the tenth and eleventh cranial nerves do not present a definite segmental arrangement.

4. The trunk ganglia of the ninth and tenth cranial nerves (gang. petrosum and gang. nodosum) when first identified are not definitely connected with the root ganglia of the same nerves, and they differ from the root ganglia in having an arrangement segmentally related to the gill arches, and possessing rudimentary sense organs.

5. The ganglia found on the rootlets of the eleventh cranial nerve are the counterpart of the root ganglion or jugular ganglion of the tenth. They do not reach the high development of the latter, though traces of them persist in the adult. They are to be distinguished from the pre-cervical ganglion of Froriep, which represents an extra spinal ganglion.

6. The eleventh cranial nerve extends caudalward into the spinal region to the third or fourth cervical segment, in some cases further; the extent and variation in the embryo is the same as in the adult. The caudalward invasion of this nerve is phylogenetic, and not ontogenetic.

7. The hypoglossal nerve in young embryos closely resembles the ventral roots of the adjacent cervical nerves, and is segmentally continuous in the same line with them. That a phylogenetic retrogression has removed the dorsal roots, which they seem to have at one time possessed, is evidenced both by the occasional presence of a Froriep ganglion and by cases in which the retrogression has gone still further caudalward, and has removed the dorsal root of the first cervical nerve.

8. The ramus descendens hypoglossi is developed in some cases before the hypoglossus has received any connecting branches from the cervical

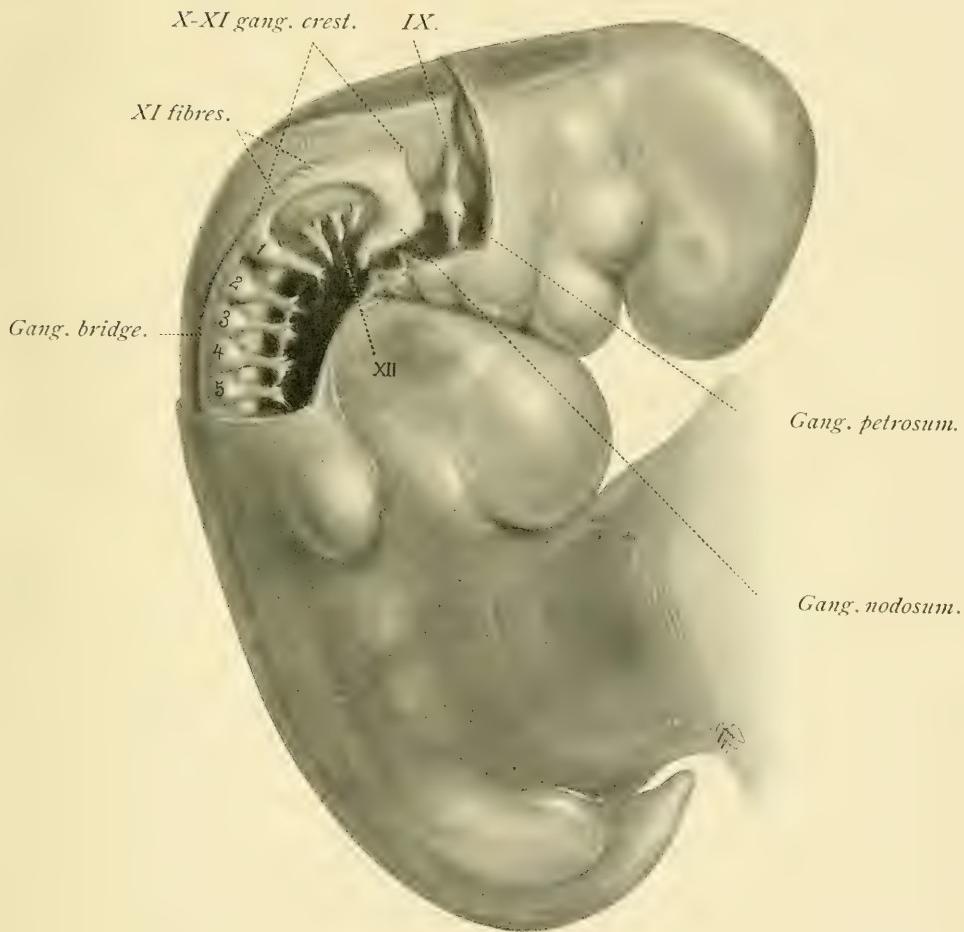
nerves; in other cases such connections are formed coincident with or before the r. descendens appears. A variable relation exists between the r. descendens and the communicating cervical branches.

9. The ventral roots of the spinal nerves are developed earlier than the dorsal roots. Similarly in the cranial nerves those portions generally recognized as motor are differentiated into fibre paths earlier than the sensory elements.

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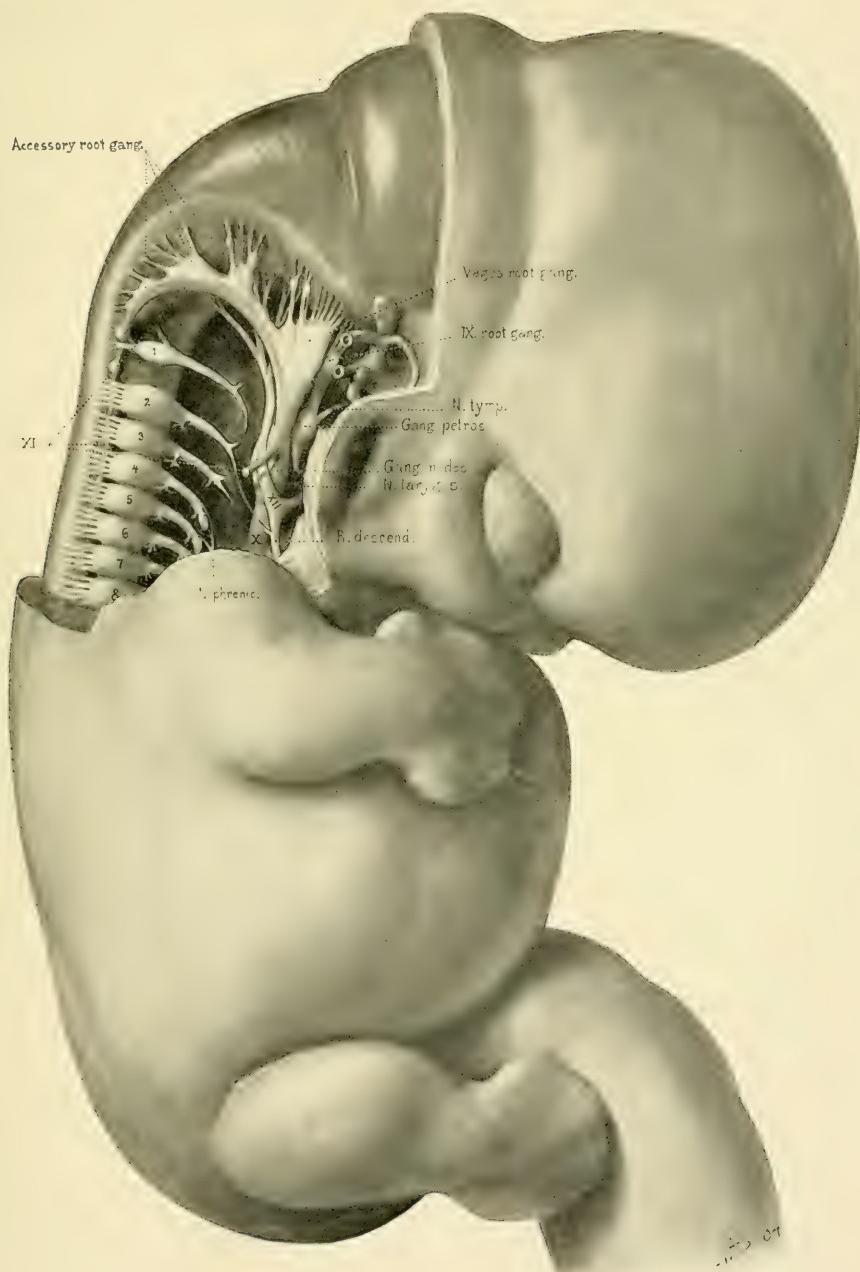


PROFILE RECONSTRUCTION OF PERIPHERAL NERVES IN THREE WEEKS HUMAN EMBRYO,
4.3 MM. LONG, MALL COLLECTION No. 148. ENLARGED ABOUT 25 DIAMETERS. THE THIRD
DIMENSION FOR THIS DRAWING WAS OBTAINED FROM A CLAY MODEL, AND THE SHADING IN
PART COPIED FROM A PIG EMBRYO OF SAME STAGE.

DEVELOPMENT OF THE OCCIPITAL NERVES

GEORGE L. STREETER

PLATE II



WAX PLATE RECONSTRUCTION OF PERIPHERAL NERVES IN OCCIPITAL REGION OF FIVE WEEKS HUMAN EMBRYO, 14.0 MM. LONG, MALL COLLECTION No. 144. ENLARGED ABOUT 25 DIAMETERS.



DEVELOPMENT OF THE OCCIPITAL NERVES

GEORGE L. STREETER

PLATE III

X root ganglion.

IX root ganglion.

Corp. quadrigem.

Cerebellum.

XI root ganglia.

*Os occip.
Atlas.*

Axis.

A. vertebralis.

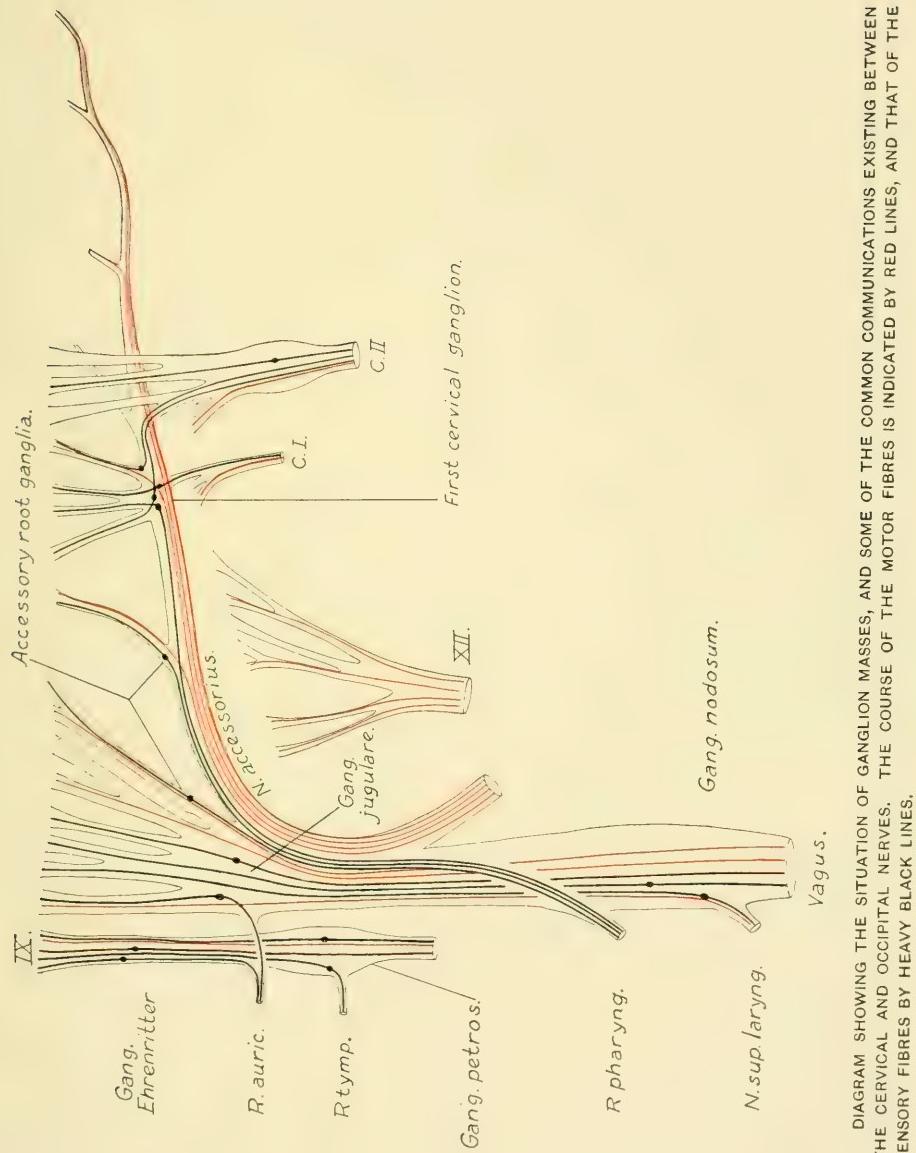
M. sternocleidomastoideus.



DISSECTION OF NERVES IN OCCIPITAL REGION OF THREE MONTHS HUMAN EMBRYO, 65.0 MM. LONG, MALL COLLECTION. ENLARGED 4 DIAMETERS.

DEVELOPMENT OF THE OCCIPITAL NERVES
GEORGE L. STREETER

PLATE IV



A FURTHER STUDY OF THE DEVELOPMENT OF THE EXCRETORY ORGANS IN *BDELLOSTOMA STOUTI*.

BY

GEO. C. PRICE, PH. D.,

Associate Professor of Zoology, Leland Stanford Jr. University.

WITH 31 TEXT FIGURES.

Much of the following work has been done in the Embryological Laboratory of the Harvard Medical School, and it gives me great pleasure to here acknowledge my many obligations to the Director, Professor Charles Sedgwick Minot, for his unvarying kindness and helpfulness. I wish also to express my thanks to Professor Oscar Hertwig for his kindness in placing the resources of his laboratory at my disposal during a four months' residence in Berlin.

In a previous paper on this subject¹ the excretory organs were described in three stages, designated as A, B and C. Of these A showed the system in an early though not in the earliest stage of development, B followed quite closely upon A, but between B and C there was a wide gap, the organs in C resembling in many respects those of the adult.

A subsequent study of better material, and of a rather full series, beginning with specimens younger than A and ending with others older than C, has revealed certain errors both of observation and interpretation, and has at the same time brought to light some new and interesting facts. It is the object of the present paper briefly to present these facts and to correct the errors. No attempt will be made to discuss the morphology of the excretory organs of the vertebrates in general.

It may be stated at the beginning that additional observations have abundantly confirmed the main point of the previous paper, namely, that in *Bdellostoma* the entire excretory system arises as a pronephros, and that from this both the pronephros and mesonephros of the adult are derived.

In the previous work, on account of the difficulty of counting the myotomes in transverse series, especially at the anterior end, recourse

¹ Price, G. C.: Development of the Excretory Organs of a Myxinoid, *Bdellostoma stouti*. *Zool. Jarb.*, Vol. 10, Anat., 1897.

was had to the spinal ganglia for the purpose of determining relative positions. It now seems best to employ the myotomes for this purpose, especially since in the earlier embryos there is an intimate relation between the myotomes and the excretory organs. It has been found that the first spinal ganglion is located between the third and fourth myotomes, so that where trouble is experienced in counting the myotomes, the ganglia may be counted instead, and the necessary calculations made.

The anterior end of the excretory system is more or less rudimentary, and the position of the first tubule is not constant, but varies both in different individuals and on the two sides in the same individual. In a few cases where its position could be accurately determined, and where there was no probability of degeneration having taken place, it occurred in segments eleven to thirteen. In one of the specimens previously studied the first tubule occurred in sections passing through the sixth ganglion. But here the preservation is not all that could be desired, and it is possible, though not certain, that this may really be the seventh ganglion, and the tubule might correspond to the myotome just back of this, which would be the tenth. But making all possible allowances, the fact remains that here the first tubule is farther forward than in any other embryo studied.

The position of the posterior end of the segmental duct has been found to vary from about the seventy-ninth to the eighty-second segment, and possibly a larger number of specimens would show still greater variation.

In general it may be said that in this animal there is a good deal of variation, both in the embryo and in the adult, a fact that should not be lost sight of in attempting to generalize from a small number of individuals.

So far as my knowledge extends Dean² is the only other person who has published observations on the excretory system in the embryos of *Bdellostoma*. From surface views alone he has described a series of segmental structures, about eighty in number, extending from the region of the neck into that of the tail, and present for a comparatively long period of the development. They are described and figured as large and somewhat complicated bodies, each extending outwards from near the spinal ganglion to the distal region of the somite, and it is suggested that they may be tubules of a pronephric nature. In sections of embryos

² Dean, Bashford: On the Embryology of *Bdellostoma stouti*. A General Account of Myxinoid Development from the Egg and Segmentation to Hatching. Festschrift zum siebenzigsten Geburstag von Carl von Kupffer. Jena, 1899.

of ages corresponding to those in which the above structures are figured I have been unable to find any such system of tubules, although on account of their size it would seem impossible to overlook them if present. In Dean's figure 134 the parts marked pronephric tubules bear a striking resemblance, even down to the fine details, to the ventral part of the muscle segments as seen in sagittal sections of an embryo of about the same age as the one from which the above figure was taken, and in the sections there is a comparatively large amount of connective tissue between the muscles which corresponds well with the space between the so-called tubules. Moreover, the muscle segments are the only segmental structures that at all correspond in size to the ones in question.

The youngest embryo studied has one hundred and one myotomes on the one side and one hundred and two on the other, a number fully equalling that of the muscle segments in the adult. There are seven or eight pairs of forming gill slits, only five of which could be made out from the surface view.

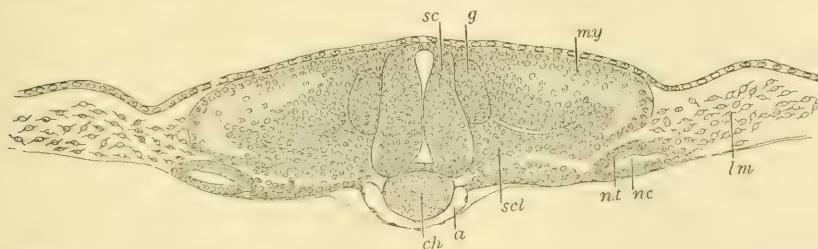


FIG. 1.—Section through the fifty-second segment of the youngest embryo studied. *a*, aorta; *ch*, notochord; *g*, spinal ganglion; *lm*, lateral mesoblast; *my*, myotome; *nc*, nephrocoel; *nt*, nephrotome; *scl*, sclerotome. There is an artificial break, shown best on the right of the figure, separating the myotome from the nephrotome and extending into the sclerotome. The sclerotome is seen to be in connection with the myotome, and on the right the lateral mesoblast is continuous with the nephrotome.

In this embryo there are neither excretory tubules nor excretory ducts in the true sense of the word, but there is an extensive system of nephrotomes from which both tubules and ducts are derived. These extend certainly from the thirteenth segment to the seventy-fourth on one side and to the seventy-fifth on the other, and it is possible they may begin even farther forward than the thirteenth. From the anterior end back to the neighborhood of the fiftieth segment they are all practically in the same stage of development, but from here on they become gradually less well developed until, in the last few segments, they appear to be just forming.

An idea of the relations of the nephrotomes to other parts may be had from Fig. 1, which represents a section passing through the fifty-

second segment, and therefore very near the middle of the body region and about five segments back of the middle of the region of the nephrotomes. The mesoblast, which alone interests us, is divided on either side into four parts, the myotome, *my*, the sclerotome, *scl*, the nephrotome, *nt*, and the lateral mesoblast, *lm*.

The myotome comes into direct contact with the ectoderm, there being no mesenchyme between them, and this is true for all the segments except the first four or five. There is no myocœl, but the centre of the myotome differs from the periphera in being free from nuclei. In the anterior segments muscle fibres are forming. Nothing of this is seen in Fig. 1, although in the section next in front, which passes nearer the middle of the myotome, some of the cells show signs of differentiation, and this is true also in four or five of the segments further back.



FIGS. 2-8.—Seven consecutive sections from the middle of one nephrotome to the middle of the next. *nc*, the nephroœel shown in Figs. 2 and 8. Fig. 5 represents the place where the two nephrotomes come together.

The sclerotome, which is formed of compact mesoblast, is still connected with the myotome, and its segmental character is further shown in running through the series, by the fact that at regular intervals the tissue here becomes much less compact. In about the last twenty-seven segments the sclerotomes have not yet been formed.

The lateral mesoblast has the appearance of loose mesenchyme, and extends outward from the nephrotome with which it is continuous. (This is shown only on the right in the figure.) In this section there is no indication of a splitting into somatic and splanchnic layers, although, as will appear later, the process has begun farther forward.

The nephrotome lies just below the outer end of the myotome, from which, in this case, it is separated by a narrow, artificial space, caused presumably by the action of reagents. It is sharply marked off from the surrounding tissue by the compactness of its walls, and it contains a rather large cavity, the nephroœel, *nc*. In running through the series the segmental character of the nephroœels is very striking, as may be

gathered from Figs. 2 to 8, representing a continuous series from the middle of one nephrotome to the middle of the next, but the segmental character of the solid portion of the nephrotome is not so apparent. This is because the nephrotomes in adjoining segments come into direct contact with each other, and some part shows in every section, just as the segmentally arranged myotomes appear in every section. However, the nephrotome becomes smaller towards the end than it is in the middle, and the point where two nephrotomes come together (Fig. 5) can quite easily be detected by the smaller size of the section and the indistinctness of the outline.

In order to gain an idea of the appearance of a sagittal section through the nephrotomes, camera lucida drawings were made of all the sections in three segments, and from these Fig. 9 was reconstructed on millimeter paper.

The proportions are here fairly accurate, but it was a question whether the nephrotomes should be represented in close contact with one another, or as having a slight space between them. It is possible, though not probable, that an actual sagittal section might even show continuity between adjacent nephrotomes.

The posterior end of one nephrotome and the anterior end of the next (Figs. 4 to 6 and Fig. 9) may be looked upon as forming a sort of short, discontinuous rod. This would be more apparent in a frontal than in a sagittal section. This remark is made because later they form an actual rod extending from one nephrotome to the next, and giving rise ultimately to the greater part of the segmental duct between consecutive tubules.

What has been said thus far in regard to the nephrotomes applies to those representing a middle state of development, those toward the anterior end being in some respects more advanced, and those toward the posterior end less advanced. As an example of the latter we may take the one in the sixty-eighth segment, a section through which is represented in Fig. 10. Here the nephrotome is attached to the myotome, although a well marked constriction has appeared between them. There is a very small nephrocel, but the greater part of the centre of the nephrotome consists of non-nucleated protoplasm which is continuous with the non-nucleated protoplasm of the myotome. There is no distinct boundary between the nephrotome and the sclerotome. On this side the last nine nephrotomes are attached to the myotomes, and a few just in front have somewhat the appearance of having been torn away.

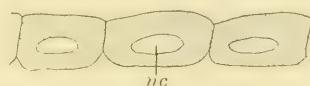


FIG. 9.—A reconstruction of a sagittal section through three nephrotomes, made on millimeter paper. *nc*, nephrocel.

Of the nine that are attached the second, third and sixth have small nephrocoels, while in the rest the center is made up of non-nucleated protoplasm. The transition between the nephrotomes represented in Fig. 1 or Figs. 2 to 8 and the one represented in Fig. 10 is gradual. Toward the posterior end the constriction between the nephrotome and the myotome becomes less pronounced than in Fig. 10. It is possible that if this embryo had been allowed to develop nephrotomes would have appeared still farther back; at least they are found farther back in older embryos.

The only striking difference between the nephrotomes in the anterior region and those represented in Fig. 1 is that in the former the nephrocoels communicate with the splanchnocœl, as may be seen in Fig. 11.

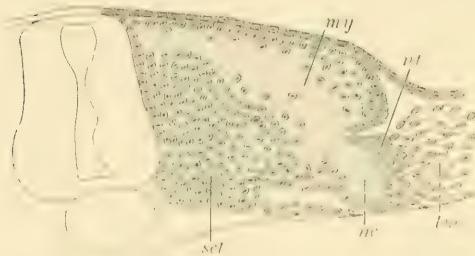


FIG. 10.

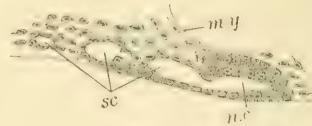


FIG. 11.

FIG. 10.—Section through the sixty-eighth segment showing a nephrotome connected with the myotome, though partly separated from it. *my*, myotome; *lm*, lateral mesoblast; *nc*, a very small nephrocoel; *nt*, nephrotome; *scl*, sclerotome. The myotome, nephrotome and sclerotome are all continuous with one another, and the lateral mesoblast is continuous with the nephrotome.

FIG. 11.—Section showing the nephrocoel, *nc*, opening into the splanchnocœl, *sc*. It also shows the way in which the splanchnocœl first appears as small, rounded cavities in the lateral mesoblast. *my*, the outline of the end of the myotome.

Here the distinction between the nephrocoel and splanchnocœl is at once apparent by the difference in the character of their bounding walls. In this region the nephrotomes come into direct contact with the myotomes and in the fifteenth segment on the right side there is actual continuity between the two.

A few words regarding the distribution of the nephrotomes. In segments nine to twelve there are cavities having the shape and position of nephrocoels, but with bounding walls less well marked than is the case with nephrocoels farther back, and it is doubtful whether they would give rise to any part of the excretory system. Nevertheless the feeling can hardly be avoided that these are rudimentary nephrotomes. On the right side in segments thirteen to fifty, and on the left in segments thirteen to forty-two the nephrotomes are in direct contact with

the myotomes, and, in the one case above noted, there is actual continuity. In segments fifty-one to sixty-seven on the right, and in forty-three to sixty-five on the left, the nephrotomes and myotomes are separated by narrow, artificial spaces. In segments sixty-eight to seventy-five on the right, and in sixty-six to seventy-four on the left, the nephrotomes are joined to the outer end of the myotome. It seems fair to suppose that in earlier stages all the nephrotomes would be found connected with myotomes, but this could be proved only by the examination of a younger embryo.

A brief account of the formation of the splanchnocœl will now be given. This arises in the lateral mesoblast as small rounded spaces (Fig. 11) which grow together, and thus form a continuous cavity on either side, and at the same time split the lateral mesoblast into somatic and splanchnic layers. (No reference is here made to the pericardial cavity.) The entire cœlom of the right side, both nephroœcels and splanchnocœl, was reconstructed on millimeter paper, and this shows that the region of most active formation is in segments nine to fifty-three. In segments fifty-four to fifty-seven it is almost absent, while back of this it is entirely absent. Fig. 12 shows the cœlom in three segments, the heavy lines indicating the boundary of the nephroœcels, and the light that of the splanchnocœl. No attempt is made to show anything beyond the outline of the cavities. It will be observed that the divisions of the splanchnocœl communicating with the nephroœcels have a sort of segmental character, and that the cavity in one segment in no case quite communicates with that in the next. Further forward, however, such communications do occur, in one instance the cavities in four consecutive segments being joined together.

In a few cases a splanchnocœlic cavity was found lying just beyond the nephroœcel, but without any communication between them. In others, as in Figs. 13 to 15, the two are seen to be breaking into each other. This may not be the only way in which the communication between the nephroœcel and splanchnocœl is established; in some cases (Fig. 1, right side) a splitting seems to begin at the nephroœcel and extend outward into the lateral mesoblast.

The next embryo to which attention will be called is between the one

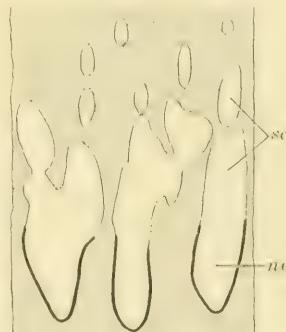
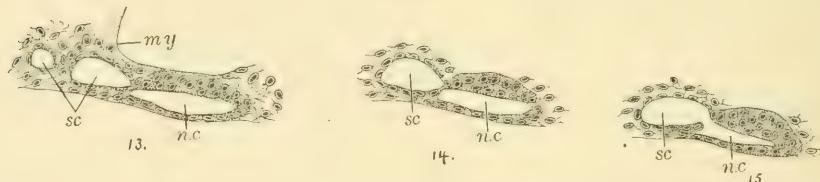


FIG. 12.—Reconstruction of the coelom in three segments of an embryo in which the splanchnocœl is in an early stage of formation, made on millimeter paper. The nephroœcel, *nc*, is bounded by the heavy line, and the splanchnocœl by the light. Nothing is shown beyond the outline of the cavities.

just described and the youngest of stage A, previously studied, but is nearer the latter than the former. It was cut in two in the region of the forty-sixth segment, the anterior part being sectioned sagittally and the posterior part transversely. There are eleven or twelve pairs of gill slits. Mesenchyme is present between the myotomes and the external ectoderm, and the sclerotomes have been converted into very loose mesenchyme, which shows no signs of segmentation. The splanchnocœl is found from about the ninth to the eightieth segment, and through the greater part of its extent is continuous from segment to segment. In the posterior region, however, it is poorly developed, and here sections are occasionally met with in which it is entirely wanting.

The excretory system extends on the one side from the twelfth to the eighty-second segment, and on the other from the eleventh to the eighty-first. Through the greater part of its extent it is no longer in contact with the myotomes, mesenchyme having grown in between, but towards



FIGS. 13, 14 and 15.—Three consecutive sections showing one way in which the nephrocoel, *n.c.*, and splanchnocœl, *sc*, come together. The section in front of Fig. 13 shows no splanchnocœl at all, while Fig. 15 is the only section in which there is a communication between the two cavities.

the posterior end of the system the ends of the myotomes gradually approach the nephrotomes, until, in about the last ten segments, the two are either in contact or are actually joined together. From the anterior end back to about the twenty-eighth or thirtieth segment the nephrocoels have either disappeared or are disappearing, by being merged with the splanchnocœl. Beyond this they are all in connection with the splanchnocœl, except the very rudimentary ones in the last four segments.

A good idea of the excretory system as found throughout the greater part of its extent in this embryo may be gained by an examination of Fig. 16, representing a sagittal section through segments forty-four and forty-five, Fig. 17, representing a transverse section through the nephrotome in segment forty-seven, and Figs. 18 to 20, three consecutive sections through the segmental duct, back of Fig. 17. The plane of the sagittal section does not quite coincide with the long axis of the body, hence the difference in shape of the nephrocoels in Fig. 16, the left or posterior one being nearer the median line than the right.

The nephrocel has increased in size, especially in its dorso-ventral diameter, and the dorsal wall of the nephrotome as seen in transverse section (Fig. 17), is arched, as if being evaginated to form a tubule. As will appear later this is true only in part. A glance at the sagittal section (Fig. 16) proves that we are still dealing with nephrotomes and that a tubule in the strict sense of the word has not yet appeared.

Extending from one nephrotome to the next is a solid rod of cells (Fig. 16, *d*), a part of the segmental duct. At one point, about half way between the nephrotomes, the duct is slightly smaller than elsewhere, and here the nuclei are much less numerous. This is brought out still more clearly in the transverse sections (Figs. 18 to 20). Fig. 19 corresponds to the point *x* in Fig. 16, and Figs. 18 and 20 are the sections

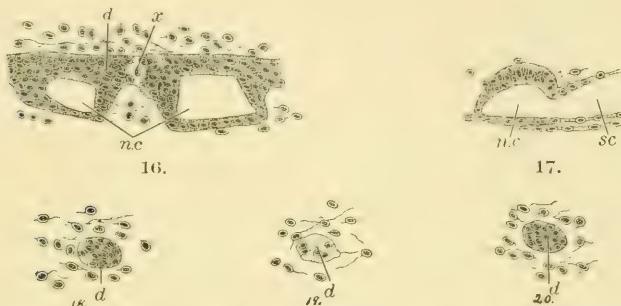


FIG. 16.—Sagittal section through the nephrotomes in segments forty-four and forty-five, the one in forty-four being to the right. *d*, forming segmental duct; *nc*, nephrocel; *x*, place where the two nephrotomes have joined together.

FIG. 17.—Transverse section through the nephrotome in the forty-seventh segment of the same embryo from which Fig. 16 was taken. *nc*, nephrocel; *sc*, splanchnocel.

Figs. 18, 19 and 20.—Three consecutive sections through the duct between the nephrotomes in the forty-seventh and forty-eighth segments. Fig. 19 corresponds to the point *x* in Fig. 16 and represents the place where the ends of two nephrotomes have united.

next on either side. The relation of the duct to the nephrotomes, and a comparison with the younger embryo suggest very strongly that it has been formed from the posterior end of one nephrotome and the anterior end of the next, the point *x* being the place where the two have united. This point of union is easily made out in almost all of the segments.

The description just given answers in all essential respects for about three-fourths of the segments, or to be exact, for all except four at the posterior end and twelve or fifteen at the anterior end. However, as we approach the posterior end, in the region where in the older embryos the tubules are rudimentary and early disappear, the nephrotomes become quite a little smaller.

In the last four segments the duct is present as a continuous rod, the outlines of which are not quite so distinct as farther forward. At four places, however, situated at perfectly regular intervals, the myotomes approach and are joined to the duct, and here the center of the duct is filled with non-nucleated protoplasm, in which is a small cavity. These can be none other than rudimentary nephrotomes. Whether they would have developed farther cannot be said, but they are interesting as indicating that the entire duct is formed from nephrotomes, for it is not likely the duct would have extended farther back, at least no case has been found where it extends beyond this point, the eighty-second segment. In the previous paper tubules were found to within two segments of the end of the duct, and as it was then thought that the duct was formed from the coelomic epithelium, and as there was no coelom in these segments it was suggested that here it had grown back independently. The present observation renders this improbable.

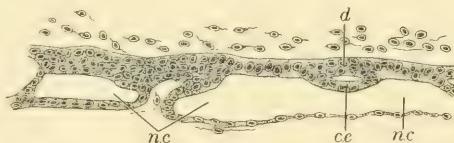


FIG. 21.—Section through the excretory organs in segments twenty-three, twenty-four and twenty-five, twenty-three being to the right. *d*, segmental duct; *ce*, coelomic epithelium; *nc*, nephrocoels. The nephrocoels in segments twenty-four and twenty-five are growing towards each other, while the one in twenty-three has met and united with the one in twenty-four. The duct does not show where the two ends of the nephrotomes have united, as in Fig. 16.

Turning now to the anterior end it remains to be shown how the nephrocoels become merged with the splanchnocœl. This is brought about simply by the nephrocoels in adjoining segments growing together, the process beginning at the anterior end and proceeding posteriorly. Fig. 21 is a sagittal section through the excretory organs in segments twenty-three, twenty-four and twenty-five. The nephrocoel in segment twenty-five, the one to the left, has not yet united with the one in segment twenty-four, although the two have approached each other; but the one in segment twenty-four has united with the one in twenty-three. From here forward sections corresponding to the one just given show no signs of nephrocoels, but if the series be followed toward the median line it is found that in the three or four sections before its entire disappearance the coelom presents the appearance of segmentally arranged cavities, showing that the fusion of the nephrocoels is not entirely completed. Between segments twenty-three and twenty-four the segmental duct, *d*, is seen to be separated by a narrow space from the coelomic epithelium.

In some of the segments farther forward this is not the case, the two being in contact or actually fused together. This union is to be looked upon as secondary.

Stages *A* and *B* previously described follow in natural sequence upon the one we have just been considering, but before proceeding to the gap between *B* and *C*, it will be well to pause for a time, and describe a certain phase in the early differentiation of the segmental duct and tubules and also notice some of the mistakes of the earlier paper.

Beginning with an embryo not a great deal older than the last, and corresponding well with the youngest of stage *A*, it is found that a constriction has occurred in the nephrotome, the most obvious result of which is to divide the nephrocoel into three parts, a dorsal part, which helps to form the lumen of the segmental duct, a ventral part, which later forms

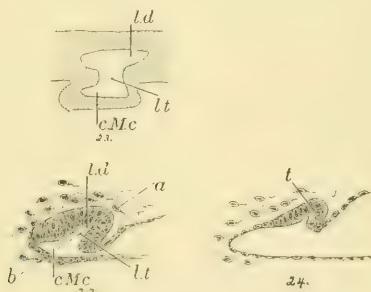


FIG. 22.—Transverse section through the tubule and duct in the forty-fifth segment of an embryo corresponding to stage *A* of the previous work. *cmc*, what will later be the cavity of the Malpighian corpuscle; *ld*, lumen of the segmental duct; *lt*, lumen of the tubule. The line *a*—*b* corresponds to the plane of Fig. 23.

FIG. 23.—Oblique longitudinal section of the segmental duct and tubule in the segment from which Fig. 22 was taken, reconstructed on millimeter paper. The plane of the section corresponds to the line *a*—*b* in Fig. 22. *cmc*, what will later form the cavity of the Malpighian corpuscle; *ld*, lumen of the segmental duct; *lt*, lumen of the tubule.

FIG. 24.—Section of a tubule in the anterior region where the nephrocoels have disappeared, of the same embryo as the one from which Fig. 22 was taken. *t*, tubule.

the cavity of the Malpighian corpuscle, and a middle part, which forms the lumen of the tubule proper. This is not apparent in a single transverse section, such as Fig. 22, passing through the forty-fifth segment, but it is brought out clearly by the study of a series of sections through a segment, and still better in a longitudinal section such as Fig. 23. This was reconstructed on millimeter paper, and represents an oblique section, the plane of which corresponds to the line *a*—*b* in Fig. 22. The constriction has affected the lower part of the nephrotome, but not to so great an extent as the middle part. A comparison of Fig. 23 with the right hand nephrotome in Fig. 16, from the younger embryo, will

help to make the matter clear. The posterior half of the excretory organs in both these embryos were reconstructed on millimeter paper. It was found that thirty segments of the older embryo occupied the same space as thirty-one of the younger, so the length of a segment is very nearly the same in the two embryos. It was found further that there was no lumen in the segmental duct of the younger embryo, but in the older embryo in every segment there was such a lumen, extending in both directions from the tubule, but more in the posterior than the anterior. The length of each division of the lumen was nearly the same as the length of the nephrocoel in the younger embryo, while the width of the tubule was less. It was thus clear that the lumen of the segmental duct had been differentiated from the nephrocoel, and had not been formed in the solid rod of cells forming the segmental duct of the younger embryo. This accounts for the fact before noted, that the lumen may extend in both directions from the tubule.



FIG. 25.

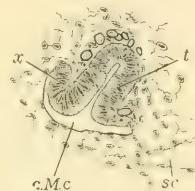


FIG. 26.

FIG. 25.—Section through a tubule in the region where the nephrocoels have disappeared in an embryo corresponding to stage *B* of the earlier paper. *t*, tubule; *sc*, splanchnocoele; *x*, part corresponding to the part *x* of Fig. 26.

FIG. 26.—Section of a tubule which has been cut off from its connection with the splanchnocoele, and from the same embryo as Fig. 25. *c.Mc*, cavity of the future Malpighian corpuscle; *t*, tubule; *sc*, splanchnocoele; *x*, part that will form the Bowman's capsule of the Malpighian corpuscle.

In an embryo a little older some of the nephrocoels, or better tubules, have lost their connection with the splanchnocoele, by the somatic mesoblast at the point where the splanchnocoele and nephrocoel come together, growing down and uniting with the splanchnic mesoblast. In general, the process occurs first in the posterior region, but it does not begin at any one particular segment and from there proceed in regular order, as may be gathered from the fact that on one side in this embryo the tubules which are closed off are in segments thirty-nine, forty-three, fifty-eight, sixty, sixty-four to sixty-six, and sixty-nine to seventy-one. Beyond this the tubules are degenerate and have almost disappeared. On the opposite side the tubules which are closed off are not quite so numerous, nor do they always occur in the same segments.

In the anterior region where the nephrocoels have disappeared the

tubules are formed as evaginations of the dorsal walls of the nephrotomes. However, it is not possible to tell where the tubules thus formed end, and the others begin. Figs. 22 and 24 represent transverse sections through tubules of the two regions in a younger embryo and Figs. 25 and 26 represent corresponding tubules in an older embryo. The resemblance in the appearance of the tubules from the two regions is apparent, notwithstanding the fact that there are important differences.

In Fig. 26 the thickening marked *x* forms ultimately the membrane immediately surrounding the glomerulus; the glomerulus itself appearing in the angle between this and the tubule. In Fig. 25 there is a similar thickening, *x*, but here it later entirely disappears, the tubule retains the characteristics of a pronephric tubule throughout life, and no glomerulus is ever formed. The attention was first called to the similarity of the two kinds of tubules in the above particular while searching for indications of the formation of glomeruli in the anterior part of the excretory organs. It is here given for what it is worth, although it might be interpreted as suggesting that formerly the anterior tubules as well as the posterior were provided with glomeruli.

By the disappearance of the nephrocoels in the anterior part of the body, and by their persistence and subsequent separation from the splanchnocel in the posterior part, the excretory system becomes quite early divided into two regions; but for a long time it is impossible to determine the exact boundary between the two, owing to the fact that there is an intermediate region of a few segments, in which the nephrocoels have neither merged with the splanchnocel nor have they been cut off from it, and there is nothing to indicate which their ultimate fate will be. Moreover, in front of this the nephrocoels do not end abruptly but become gradually shallower and shallower. Later the boundary becomes definite, but it is not constant for different individuals, nor is it always constant for the two sides in the same individual. The best that can be said is that it is in the neighborhood of the thirtieth segment.

Corresponding to the above described regions there are two kinds of excretory tubules, those in the anterior region retaining the characteristics of pronephric tubules throughout life, while those in the posterior region assume the characteristics of mesonephric tubules. These terms would be employed in speaking of them if it were not that it would lead later to the contradictory expression, "the mesonephric tubules of the pronephros." For this reason the first will be spoken of simply as the open tubules, and the second as the closed tubules.

Of the closed tubules those in about the last eighteen or twenty segments degenerate (the number not being constant), while the remainder

persist, and all except a small number at the anterior end become mesonephric tubules of the adult.

Both of these points were noted in the previous paper, but it was there stated that in the oldest embryo of stage *B* the tubules had disappeared in the last nineteen segments. A subsequent very careful examination proved this to be a mistake. They have disappeared in the last eleven segments, but in the next eight they are still present in a rudimentary condition. Their entire disappearance does not occur in this region until quite a little later.

In the earlier paper the statement was made that the series was sufficiently complete to enable one to say that the mesonephric tubules of the adult were derived from tubules which in the embryo arose as pronephric tubules. The opinion has been expressed by some authors that the evidence given was not sufficient to justify the assertion, and it is not to be denied that, on account of the wide gap between stages *B* and *C*, the objection may have been reasonable. But an examination of specimens both older and younger than those before studied, as well as of quite a complete series between *B* and *C*, has proved the truth of the statement. In no case in the region of the mesonephros of the adult is there the slightest indication of the disappearance of one set of tubules and the appearance of another, but on the contrary the first tubules to appear may be traced into what can be none other than the mesonephric tubules of the adult.

Wheeler³ objected further on the ground that in a young specimen of Myxine Mass found mesonephric tubules forming in the posterior part of the body independently of the duct. As a matter of fact Mass⁴ describes and figures two well developed Malpighian corpuscles, one with and the other without a tubule, neither of which is in connection with the duct. He does not state, however, whether these occur in segments in which later mesonephric tubules are invariably present, and until this is known it seems at least possible that the structures in question may be degenerating instead of developing tubules. However it may be in Myxine in Bdellostoma in a few cases very small, degenerating tubules have been found far back in the body, independent of the duct. But here a comparison with other tubules in the same embryo, as well as with embryos both older and younger, shows that these have been

³ Wheeler, W. M.: The Development of the Urinogenital Organs of the Lamprey. Zool. Jahr., Vol. 13, Anat., 1899.

⁴ Mass, Otto: Ueber Entwicklungsstadien der Vorniere und Urniere bei Myxine. Zool. Jahr., Vol. 10, 1897.

pinched off from the duct and would soon entirely disappear. As a rule the degenerating tubules do not become thus separated from the duct.

In the earlier paper the statement was made that the excretory system developed from behind forward. This was based on two facts; first, in passing from the anterior end backward through a number of segments in both stages *A* and *B*, both the duct and tubules became gradually better and better differentiated; and second, in the older embryo the system extended a little farther forward than in the younger. From the last it was assumed that if the younger embryo had developed the system would have extended as far forward as in the older, but this does not follow, as is proved by the examination of a larger number of embryos. The above opinion was further strengthened by the observation that the first tubules to be cut off from the connection with the splanchnocoele were at the posterior end. From the material at hand it cannot be determined which are the first nephrotomes to appear although it would seem that those at the posterior end are the last. The formation of the excretory system proper seems to begin in a good many segments at about the same time, but the development lags behind at the anterior end, and this gives rise to the appearance in certain stages of this being the youngest part.

The mistake of looking upon the anterior end as the youngest part led to the more serious error of supposing the nephrotomes were pockets formed from the unsegmented mesoblast; for at the anterior end where the tubules were apparently just appearing there were no indications of nephrotomes, the tubules being connected with the unsegmented body-cavity. Then in passing back, as the tubules became better and better developed they were connected at first with very shallow pockets and then with deeper and deeper ones. We now know that this appearance is due not to the formation of segmentally arranged pockets, but to the disappearance of splanchnocoels. In a review Felix⁵ pointed out the probability of this error.

In what was supposed to be the youngest part of the system the segmental duct was found to be in connection with the coelomic epithelium. This union is secondary, but it was then thought to be primary, and it was supposed that the duct in all parts arose as thickening of the coelomic epithelium. The theory was strengthened by finding the duct in connection with the coelomic epithelium in one individual in some of the

⁵ Felix, W.: Die Price'sche Arbeit "Development of the excretory organs of a Myxinoid (*Bdellostoma stouti Lockington*)" und ihre Bedeutung für die Lehre von der Entwicklung des Harnsystems. Anat. Anz., Vol. 13, Nos. 21 and 22, 1897.

posterior segments. But in younger embryos the duct has since been found in sections where the splanchnocœl had not yet been formed.

In the anterior region the duct is occasionally either partially or entirely absent between two tubules. This is to be looked upon as due either to degeneration or to the failure of the duct to develop. In an extreme case, in an embryo of stage *B*, there was a break on one side between tubules one and two, five and six, seven and eight, eight and nine, and fourteen and fifteen, and on the other between one and two, five and six, and nine and ten.

We now come to the differentiation of the pronephros of the adult, the most important steps of which occur in the stages between *B* and *C*, and were therefore not seen at all in the previous work.

In the oldest embryo of stage *B* the anterior end of the body was injured, so that the position of the anterior end of the excretory organs could not be accurately determined, but it was estimated that the system had disappeared in about nine segments. It is true that some of the anterior tubules may degenerate, but in no case in the material in hand, where the point could be accurately determined, can it be said that so many as nine have disappeared, so that the above estimate must be considered erroneous. What was said regarding the pronephros in embryo *C* was of rather a tentative nature, the preservation not being of the best, and it being impossible to work out the structure satisfactorily.

In the youngest embryo before studied the gill-slits were found well forward in the head region, while in the oldest they were well back in the body region, occupying the same position as in the adult. On entirely insufficient evidence it was assumed that the change had been brought about by slits degenerating at the anterior end and new ones being added at the posterior end, and that, in the course of development a good many more slits appeared than were present in the adult. Dean⁶ has shown that this is wrong, the first slits formed being permanent, but shifting their position from the head region to the body region. This change in the position of the gill-slits seems to be the cause of an important change in the excretory system. At all events, as the gills become shifted farther and farther backward the anterior tubules become crowded closer and closer together, until finally they form a small, compact body, the pronephros of the adult, in the region a little posterior to the thirtieth segment. The crowding affects all of the open tubules,

⁶ Dean, Bashford: On the Development of the California Hagfish, *Bdellostoma Stouti*, Lockington. (Preliminary Note) Quart. Journ. Micr. Sci., No. 158. Vol. 40, 1897.

and a small though variable number of the closed tubules as well. At all times the anterior end of the excretory system is a little back of the posterior end of the branchial system. For quite a long period the history of the differentiation of the pronephros of the adult is simply a history of the crowding together of the tubules, as will appear from the following account of the organ in a series of embryos of different ages. Before beginning, it may be stated that in all cases every section of the parts described was drawn, different colors being used for the different tubules. This was highly advantageous in all cases, and was absolutely necessary in the older and more complicated specimens. The arrangement is not usually exactly the same on the two sides, but unless there is some really important difference only one side will be described.

The first embryo of the series is a little older than the oldest of stage A, and the process of crowding together of the tubules has just fairly begun. The first tubule is in the sixteenth segment and is not connected with the rest of the system. In segments sixteen to twenty-four,

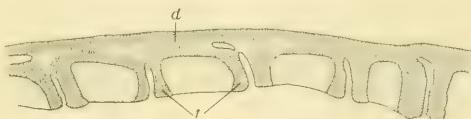


FIG. 27.—Sagittal section through the anterior end of the excretory organs in an embryo in which the tubules are beginning to be crowded together, formed by the combination of two adjoining sections. There are here five tubules in the space of three segments. *d*, segmental duct; *t*, tubules.

that is in nine segments, there are twelve tubules. Back of this the position of the tubules has not been affected. If all were arranged segmentally the first tubule would be in the thirteenth segment. The first three or four tubules are not so well developed, as the following, and look as if they might be in the process of atrophy, although this is not at all certain. Fig. 27 is a combination drawing made from two sections, showing five tubules in the three segments, twenty to twenty-two. There is quite a little space as yet between the tubules. The segmental duct is almost without a lumen, but a little further back the lumen begins, and continues with only a few interruptions to the posterior end of the duct. However, the duct does not open to the exterior.

In the next embryo the first tubule is in the twenty-second segment, and in the six segments, twenty-two to twenty-seven, there are fifteen tubules. They are not evenly distributed, being more crowded in the anterior part than in the posterior. But in no case are two tubules in actual contact with each other. One or two at the anterior end are quite rudimentary, and there was some doubt as to whether they actually

represented tubules. All are connected with the segmental duct. If they were arranged segmentally the first would be in the thirteenth segment. This embryo was sectioned transversely, and it could be determined accurately that the tubules in the thirtieth segment were the first to be closed off. However, those in the twenty-ninth segment looked as if they might be closed off later.

The first tubule in the next embryo is in the twenty-fourth segment. It is not connected with the rest of the system and is separated by quite a wide space from the second, which is in the twenty-fifth segment. In segments twenty-five to thirty there are sixteen tubules, and of these the first ten, which are in the space of three segments, are so closely crowded as to be almost if not quite in contact with one another. If all were arranged segmentally the first tubule would here be in the fourteenth segment.

In the next embryo there are seventeen tubules in the four segments twenty-seven to thirty. Of these, the first thirteen are in so short a space that there is not room for them to stand in a row one behind the other, and some are beginning to be crowded to one side—a process which is carried much farther in older embryos. A comparison of Fig. 28, taken from this embryo, with Fig. 27, shows how much closer together the tubules are here than in the younger embryos. The coelomic opening of most of the tubules shown in Fig. 28 are found in other sections. The segmental duct is no longer straight, and so does not show in the full length of the figure.

The next embryo is particularly interesting, because here the crowding process has affected not only all of the open tubules, but the three anterior closed tubules as well. The posterior limit of what will be the pronephros of the adult may be placed, with some degree of probability, between the third and fourth closed tubules, for at this place the segmental duct shows some slight indications of degeneration. Owing to an injury in the anterior region of the body, it is not possible to determine the exact position of the pronephros, but it occupies the space of a little less than two segments. There are in all twenty-one tubules, eighteen open and three closed. The first and second tubules are connected with each other, but not with the rest of the system. The three closed tubules have the same structure as those farther back, and are in direct line with them, but they occupy the space of only three-fifths of a segment, and the third slightly overlaps the second. Just at the point where the anterior closed tubule joins the duct, the latter bends downward, backward and slightly outward, and then turns and runs again forward, thus forming a sort of s-shaped bend. In this way some of the

open tubules come to lie ventral to the closed tubules, and also slightly more lateral, and a transverse section may show parts of one or even of two closed tubules, and at the same time parts of several open tubules. Fig. 29 represents such a section. The pronephros now forms a prominent body projecting into the coelom. The segmental duct is cut through its posterior bend, and four open tubules are seen to be in connection with it. By running along the series the lumen of these may all be traced to a connection with the duct on the one hand and with the coelom on the other. Above, one of the closed tubules is cut through its full length, but the lumen appears only at the point where it joins the duct. Farther on in the series it may be traced to a connection with the

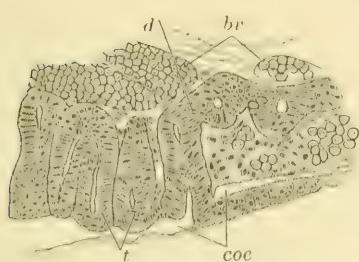


FIG. 28.



FIG. 29.

FIG. 28.—Sagittal section showing parts of seven tubules, and illustrating the way in which they become crowded together. In studying the series all the tubules are found to open to the coelom. *cæ*, coelom; *bv*, blood-vessels; *d*, segmental duct; *t*, tubules.

FIG. 29.—Section of a pronephros showing the two kinds of tubules, four open tubules below and a closed tubule above. *c.M.c*, cavity of the Malpighian corpuscle; *d*, segmental duct, here shown in two places; *gl*, glomerulus of the closed tubule; *t*, tubule.

cavity of the Malpighian corpuscle. There are places in the pronephros where the segmental duct has no lumen. This is the first embryo in which the segmental duct was observed to open to the exterior at its posterior end.

In an embryo slightly older than the last, the pronephros was not thoroughly studied, but it was observed to be more sharply set off from the mesonephros than in the last, although the segmental duct was still continuous between them. It occupied parts of the thirty-third and thirty-fourth segments. The number of closed tubules was not determined, but two glomeruli were seen to be in very close contact with each other.

The next embryo is somewhat younger than embryo *C* of the previous

paper. The pronephros on the right side is in segments thirty-two and thirty-three. There are in all twenty-one tubules, and of these the first three have no connection with the segmental duct, while the last three can be traced to a connection with Malpighian corpuscles, and must therefore represent closed tubules. On the left side the pronephros is in segments thirty-three and thirty-four, and there are here nineteen tubules instead of twenty-one. The first two are not connected with the duct, and two instead of three represent closed tubules. If the tubules were arranged segmentally, the first would be in segment thirteen on the right side and sixteen on the left, while the anterior closed tubule would be in segment thirty-one on the right and thirty-three on the left. On both sides the pronephros is connected with the mesonephros by the segmental duct, and in neither case does it occupy the full width of two segments.

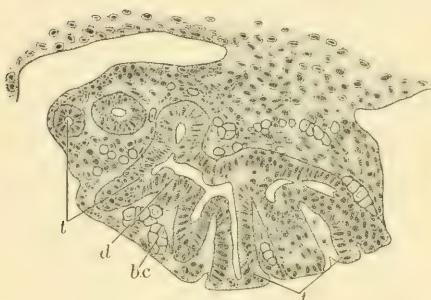


FIG. 30.

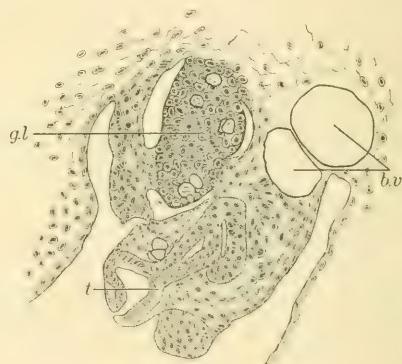


FIG. 31.

FIG. 30.—Sagittal section through the pronephros showing several open tubules, all but two of which are cut in longitudinal section. In this embryo the limits of the pronephros are definitely established. *bc*, blood corpuscles; *d*, segmental duct; *t*, tubules.

FIG. 31.—Transverse section through the pronephros of the oldest embryo studied, showing one of the open tubules, *t*, in the process of branching. *bv*, blood-vessels; *gl*, glomerulus.

Fig. 30 represents a sagittal section through the left pronephros. Several tubules lie in about the same plane and are cut longitudinally. Their connection with the segmental duct is shown. Two are cut transversely. Farther on in the series these also join the duct. The section is not near enough the median line to show any of the glomeruli.

In the last embryo to be considered, which is some little older than the embryo *C*, the exact position of the pronephros could not be determined, owing to an injury farther forward, but it is located in the

pericardial cavity, in a position corresponding to that of the adult. There are on the left side twenty tubules, two of which can be traced to a glomerulus and must therefore represent closed tubules. Some three or four of the open tubules have begun to branch, as is shown in the one in Fig. 31. This is the beginning of a process which results in the formation of the scores of pronephric funnels found in the adult. The two closed tubules are small and insignificant, but the glomerulus to which they go is large and well defined, and has something the appearance of having been formed by the fusion of two glomeruli. At one point, shown in Fig. 31, the cavity at the side of the glomerulus, the cavity of the Malpighian corpuscle, is almost in communication with the *cœlom*. This is interesting as suggesting that in some cases the communication might actually occur, in which case the glomerulus would be virtually in the body-cavity. However, this is to be regarded as only a passing remark, and not as suggesting a homology between this glomerulus and the glomus of the pronephros in other forms. Nothing of the kind occurs on the other side, nor has it been observed in any other embryo. There is a complete break in the segmental duct between the pronephros and mesonephros. In the pronephros itself the duct is distinct and the lumen well defined through about half its course on one side and two-thirds on the other. In the rest it is solid, and in places quite small. It may be remarked that no case has yet been observed where the lumen of the duct is continuous throughout the entire pronephros.

A point that has not been worked out is the manner in which the segmental duct becomes shortened as the tubules are crowded together. The bending of the duct previously described accounts for part of it though hardly for all.

The account of the pronephros just given does not fit in well with Mass'⁷ description of this organ in a young specimen of *Myxine*, where there is no segmental duct; but the difference is hardly to be accounted for on the supposition of errors of observation in either the one case or the other, nor is it likely that later the pronephros of *Bdellostoma* would come to be more like that of the young *Myxine*. An examination of the pronephros of the adult *Bdellostoma* reveals the presence of a true segmental duct, although it may not be continuous through the entire organ, while, judging from the published accounts of the pronephros of *Myxine*, the presence of a true segmental duct here would seem to be very doubtful. In the light of this the above discrepancy is not surprising.

⁷ Mass, Otto: I. c.

The presence of the excretory organs in segments where later the gill slits were found, was formerly thought to constitute a resemblance between the excretory system of *Bdellostoma* and that of *Amphioxus*, in which the position of the excretory system and branchial system coincide, but this opinion is no longer held.

WILHELM HIS.
HIS RELATION TO INSTITUTIONS OF LEARNING.
BY
FRANKLIN P. MALL.
Professor of Anatomy, Johns Hopkins University.

The ancient science of anatomy has been perpetuated and extended during the many centuries of its existence by great men who have dedicated their lives to it. The list is a long one for the development of the science has been slow and progressive from the earliest ages to the present time; we find in it on the one hand, some of the names of the greatest who have ever lived—Aristotle, Vesalius—on the other, the names of those who rank as leaders of a generation, Bichat, His.

Undoubtedly the reason for the continuous progress of anatomy through so many centuries is that man has always shown an interest in a knowledge of his own structure and, in turn, that this knowledge has been of great service in battling for rationalism against mysticism in many directions. But in order that a science may progress, it must be in the hands of able men with the highest ideals and with inexhaustible zeal. The full benefits of a science cannot be obtained from its literature alone; it is well known that countries without leaders in a science live almost in ignorance of it. No science will develop to its fullness unless it is represented by men who are great enough to grasp the science as a whole and broad enough to understand its relation to cognate sciences as well as to the needs of a civilized community.

A man of this caliber was Wilhelm His. He was born of a distinguished family which felt that it owed much to the community and therefore educated its children for a strenuous life to be dedicated to the community. He was in no sense a self-made man though he had in him the qualities to make one; his powers were fortunately developed under the best possible conditions. His father taught him, by example, simplicity in living, clearness of thought and seriousness of life. His mother, who died while he was still a youth, guided his education with the greatest care, laying much stress upon a good command of German. In a pri-

vate school in a country town, he received a training so rigorous that it makes one shudder to think of it. At the age of thirteen, he returned to his home in Basel and entered the Gymnasium from which he graduated five years later, in 1849.¹ During his years in the Gymnasium, His rated only as an average pupil, for his bent toward physics and natural history was great, and what spare time he had was devoted to these subjects. He also photographed a great deal, the art then being still in its infancy; he constructed his own camera and made his own plates. In those days the professors of the University taught also in the higher classes of the Gymnasium (the Pedagogium, as it was called), and it was from them that he received his greatest inspiration. Especially grateful was he to the Professor of German for a thorough training in the use of the German language in which he had received such a good start in his father's house.

Throughout life the good command of language he had gained and the marked technical ability he had acquired were of the greatest use to him in furthering the cause of anatomy; it is interesting to note that these powers were developed long before he entered the University. It was fortunate for him and for anatomy that he came under the influence of able men early in his career.

Most of his student friends had decided to study law and naturally His thought that he must accompany them. His inward feeling was drawing him towards natural history, but he felt that one's inclination is to be viewed as a forbidden fruit and therefore he had nearly decided to go with the stream of his fellows. Before taking the final step, however, he consulted his superiors, one of whom was Winscheid, later one of the foremost jurists of Germany. Winscheid advised him to follow his own bent and this carried him into medicine.

As soon as he became a student of medicine in the university, he found himself free to follow his own inclinations. For the first few years he devoted most of his time to the sciences. Anatomy, physiology, natural history, psychology, chemistry and geology were studied at Basel and Bern under a variety of men. He soon learned that for the student, the quality of the teacher is more important than the subject taught; in order to have a great teacher and the important subject together, he decided to study anatomy with Johannes Müller, in Berlin.

The lectures of Müller were a revelation to him and he felt for the first time the inspiration of a strong personality. His learned from Müller and later from Remak, how great an influence a teacher can have upon a pupil, when, as an authority, he presents his own line of work. Then the teacher and pupil stand upon the common field of nature. The

power of the successful explorer is most stimulating. It encourages the pupil to attack open problems, and, if he can, he formulates questions and takes his position towards them. Thus Müller's presence worked upon His and unconsciously the latter soon found himself in the library studying Müller's monographs.

His also pursued a course in embryology with Remak in Berlin. The subject was then a new one, but it made a profound and lasting impression upon the young student for it showed the relation between histology, embryology and comparative anatomy. To Remak, His owed more than to Müller, for Remak's teaching helped him to formulate problems which occupied him during the following half century; his efforts to solve them, have, in many respects, changed the aspects of anatomy.

How different is the study of medicine in Europe from that in America! There freedom reigns and students wander from place to place being controlled only by a fairly rational system of examinations in case they wish to graduate. Weak students fall out, for there is no cram system to drive them onward; able students select great men as teachers and thereby develop themselves and become stronger. So His, soon tiring of the sterile lectures in practical medicine, turned his eye towards a new star which was beginning to illuminate the medical world and wended his way to Würzburg to study with Virchow. During one of Reinhardt's lectures, His had happened to see Virchow's great paper upon connective tissue which had just appeared and it made so great an impression upon him, that he at once decided to go to Virchow. He was then far from prescient of the fact that in a few years he was to take an important part in the great discussion which Virchow had opened.²

In the early fifties, the University of Würzburg was at the beginning of that rapid rise coincident with the appearance there of a brilliant band of young professors. The change was brought about largely by a reformer, Professor Reinecker, through whose personal efforts Virchow and Kölliker were secured. It was the good fortune of His to enter this atmosphere; a select faculty was chosen by a talented student. He entered Würzburg at the beginning of his fourth year of medical study and began to work in the clinics. He soon found that the laboratories attracted him more than the clinics and during three semesters much time was devoted to practical chemistry; the weekly meetings of the Scientific Society always found him present to hear of the new medicine from Virchow and Kölliker.

For a time he attended regularly a kind of journal-meeting at Kölliker's house where a variety of scientific subjects were discussed. There

he became acquainted with Ludwig's physiology which was then heterodox and called forth severe criticism. Ludwig, very critical towards others with pupils who were more so, kept the camp stirred up pretty well; this had the right effect upon his critics (His included) for they studied the new physiology. Converts were made at a rapid rate and before His had reached middle age, he found that both Virchow and Ludwig were very orthodox; the scientific world had come to them.

In Würzburg, a great opportunity came to His—Virchow at once set him at work in a good field to answer fundamental questions. Probably the most important step in the life of a scientist is to be started aright in research after a good preliminary training. The earlier this is done the better. His was twenty-one years old, mature for his years and he was given every opportunity to use all of his ingenuity and strength in answering important questions. Henle and Virchow had crossed swords, but this did not make the great pathologist try to enslave the mind of His in order to win a victory, but now as so frequently afterward, a desire to know and to understand were Virchow's only considerations in directing a pupil in his work. The results of His were not what Virchow had anticipated for they showed that Bowman's corneal tubes and Coccius' serous spaces were artifacts and had nothing whatever to do with the system of connective-tissue spaces described by Virchow. But His was encouraged to continue and the monograph upon the cornea which he published a number of years later has proven to be a standard until the present day. His had been in the arena and had shown his prowess; he had originality and strength as well as training from a great master and henceforth during all his life he was to win victory after victory for science.

It was still necessary for His to take his medical degree and as was customary with many at that time, he proceeded to Prague and Vienna to study with the great clinicians there. At the end of a year he returned to Basel and passed his examination with the highest mark.

The young student had received the best from his home, the school, the gymnasium and the university of his native city, had wandered for four years studying at famous foreign universities receiving information and inspiration from the greatest masters—Müller, Virchow, Kölliker and many others—and had now returned to Basel to receive his degree. How much longer must we wait for similar privileges in America?

The following three years of leisure and lack of responsibility, those in which the real stuff in a scholar is tested, were devoted to a continuance of the work already so well begun.³ Part of the time was passed in his private laboratory, one of the years being broken by a journey to

Paris, another by a visit to Berlin. Throughout this period he associated only with the best, for there was a never-wavering desire in him to devote his life to science. During this time he became a Privat docent in the University of Basel and gave regular lectures there on histology.⁴

In most of the continental universities any person approved by the faculty may teach and thus new blood is constantly being infused long before a fogey or a dog in the manger has been removed by a beneficent Providence. There is thus maintained a constant competition for better teaching within the walls of the university, and strong young men are brought to the front. If under these conditions a young scientist takes deep root without being spoon-fed or coerced, and commands a broad field, adding to its borders, the greatest assurance has been given that he will remain active and productive until he is three score and ten. In America, we frequently find recent graduates who tell us that they would follow an academic career if their future were assured as far as salary is concerned. Little do they realize that this attitude of mind alone should exclude them absolutely from such a career. Unfortunately, we seem to have some university presidents who are as easily deceived by such "scientists" as the public is by a Mesmer. Within a year, I have known of a president who, when seeking a great anatomist for a rich university, selected a man who had done no scientific work whatever and had never been tested, simply on his own assurance that he would "try to do something."

In 1857 the chair of anatomy at Basel became vacant, and, as is customary, the faculty sought the best available man. They found in His an able earnest young scientist of the best training, tested through freedom and research, whose work had been continued with increasing successful results through the three years of leisure following his advancement to the doctorate. A man who had studied a subject for its own sake⁵ and had contributed to it, was more likely to represent it well than one who had studied it for other rewards. Only too often do we see scholars whose work is good and imitative, but not profound, shift from one thing to another in order to keep before the public in an upward career; they find themselves sterile at forty and have to be shelved in some good berth as an active "pensioner," at fifty. To avoid this danger, great productive men must sit in faculties, for they alone are able to recognize genius in a young man.

When the Chancellor of the University informed His of his appointment as professor of anatomy and physiology, he said: "We have thrown you into the water, learn to swim;" and the remark was appropriate, for the new professor had never served an apprenticeship in the teach-

ing of either anatomy or physiology. Often did he seem to sink but he always came to the surface again, for he had overcome great difficulties before—he was independent. After all, research is to the problem of teaching as manoeuvres are to a battle.

The power in His now blossomed and bore fruit. With his great background he was able to construct strong courses, marked by his individuality, and soon he was to influence the teaching of anatomy the world over. The attitude of the comparative anatomist he had learned from Johannes Müller, that of the histologist from Virchow and Kölliker, but that which made the greatest and most lasting impression upon him was the attitude of the embryologist which he learned from Remak. The histological work begun with Virchow was continued and soon extended to include the lymphatic system. In 1862 he published a paper on lymph radicles, advocating a closed lymphatic system, by all odds the best paper on that side of the question which has ever been published.

During this time, his embryological studies were also actively prosecuted for he had learned of their great value in histology from Remak. The classic object, the chick, as well as the structure of the ovary was studied again. That a plan underlay his work was very apparent, but no one dreamed of its magnitude until the publication of his academic program in 1865, entitled "Die Häute und Höhlen des Körpers." In this paper he gives the key by which the genetic relation of tissues can be ascertained and following it faithfully, he made one discovery after another. The "Program" is now incorporated with our science; it proved to be really a program for anatomy, and it was fitting that it should have been reprinted as the last paper during His's editorship of the *Archiv für Anatomie*, nearly forty years after its first publication. It certainly must be gratifying to a scientist to see his early dreams so well realized before his work is over. As His said of Bichat, "It is a mark of genius to see great truths in a relatively small number of observations."

The great contribution to anatomy during the eighteenth century was the discovery of the tissues, the conception of which received its full development in the general anatomy of Bichat, published in 1801. Cellular tissue had been gradually making its way during that century and among others, Haller was trying to see in it some unit of organization, but the cell of Haller proved to be only a connective-tissue space and the all-important fiber was viewed by most anatomists as an artificial production. But the doctrine of cellular tissue was the foundation of general anatomy and this in turn that of histology which, through embryology, has given us modern anatomy.

During the nineteenth century we see three great steps in anatomy,

general anatomy, associated with the name of Bichat, the cell doctrine with that of Schwann, and histogenesis with that of His. Compare the great text-books of anatomy of 1790 with those of 1810, those of 1830 with those of 1850 and those of 1880 with those of 1900 and the reasons for this statement will be apparent.

At the time His began his embryological studies the plan of the development of the vertebrate body, as enunciated by Von Baer with Remak's classification of tissues, had been accepted generally. But the work of Remak was incomplete and in some respects unsatisfactory as, for instance, his conclusion regarding the development of the nervous system, the central portion of which he believed arose from the ectoderm and the peripheral portion from the mesoderm. This and other defects were corrected by His who made a new classification of tissues and germ layers which differs more from Remak's classification than this in turn did from Von Baer's.

In the "Program" His also showed that there is an embryological foundation for Bichat's classification of membranes since they are related directly to the germ layers. Further, he extended the conception of the serous spaces to include the vascular system. All of the serous spaces arise in the mesoderm, and His showed that they are lined with a special kind of cell, designated from the time of his paper on, as endothelial.

After this, his greatest work was histogenetic—witness for instance his studies upon the nervous system and upon the development of the blood-vessels. Contribution after contribution was published upon histogenesis. During the last years of his life he often complained to me that his time was short and that it was necessary for him to make haste in order to round up his work.

Thus His continued to be active until he was over seventy years old; his final papers, "although fragmentary," are of the highest quality. In his great paper upon the angioblast he gave his latest classification of tissues from an embryological standpoint⁷ and stated in conclusion that the riddle which had interested him so much still remained unsolved. Similarly, in his last monograph upon the nervous system, he wrote: "The notes are not to be considered as a report of a finished research, but they only indicate the line of work which must be followed by united effort⁸ in order that a better understanding of the structure of the brain may be gained." He saw more and more clearly that many hands are required to survey these great fields and during the last twenty years of his life, thought much about the organization of research. He lived to see his efforts in this direction crowned by the establishment of a com-

mittee for the study of the brain by the International Association of Academies, of which more presently.

In 1872, the chair of anatomy at Leipsic became vacant and through the efforts of Ludwig, His was secured. Professor Ludwig told me that it was by no means the unanimous will of the faculty to call His, for in general he was not well known, nor was he considered a good teacher. But Ludwig knew his man for he had found in His the strongest opponent of his notions regarding lymph radicles; furthermore, he was fully able to appreciate the author of the great academic program on "Häute und Höhlen." For over thirty years Ludwig and His were colleagues, consulting each other almost daily, an ideal relation for great scientists.

The work of His had branched in many directions at Basel, but it grew with increased vigor at Leipsic, for here he had all the material aid he could desire. Photographer, modeller, mechanic, technician, artist and others were at his command. A new laboratory, which proved to be a model, was built according to his ideas. Each of these factors was to play a part in the campaign he was conducting and it was soon seen how his hands were thus extended.

Through the better technical assistance, His increased his productivity, for his plan was broad enough to use it to the greatest advantage. The members of his staff, however, were never subject to his orders in their scientific investigations, nor did he ever use "research assistants" for his ethical standard would not permit such employment of scientists. That a great man can increase his productivity enormously without the questionable use of young colleagues is shown by example in the life of His. Furthermore, his plan of organizing research is one of the best from both the ethical and the scientific standpoint.

The microtome which His had invented in Basel was now perfected and better serial sections were made of embryos of different classes of vertebrates than ever before. The great monograph upon the chick had just been published and smaller but equally important papers appeared upon fishes; these were soon to be followed in 1880 by the first part of the monumental work upon human embryology.

Throughout his embryological work His was constantly interested in the broader problems and he was among the first to view development from a mechanical standpoint. His views and general plans were brought together in 1874 in the classic, "Unsere Körperform" dedicated to his new colleague, Ludwig, appropriately for in it the subject of development is presented from a physiological standpoint. These views were immediately antagonized to the utmost; when arguments failed, his opponents resorted to ridicule, but, in general, His held his

own and continued a forward course. He now became the leading advocate of the theory of mechanics in development and it subsequently tinctured all of his papers. Although he made no experiments upon the growth of animals, he must be viewed as one of the pioneers of the new science of experimental morphology.

Throughout His's embryological papers we see that he believed that the form of the animal body, as well as of its organs and tissues, is due to mechanical influences of the structures upon one another. This conception was often erroneously construed as meaning that there is a mechanical cause for growth, but His repeatedly denied this. "The stimulant which causes cells to multiply cannot be traced to mechanical influences." His mechanical conceptions of development are comparable with the study of the mechanics of the circulation rather than with that of the evolution of the heart. When analyzed, it appears to me that his mechanical conceptions are related principally to the wandering of tissues and organs in development. One of the best examples of His's ideas is his conception of the growth of nerve fiber from the central cell to the periphery, where, through secondary connections, it makes itself fast to its end organ. With this conception, we can understand and picture to ourselves the formation and the infinite number of variations of the peripheral nervous system. Other examples may be found in the wandering of the diaphragm and in the metamorphosis of the branchial arches. Mechanical influences must guide these structures to their fate. And finally, great masses of tissue wander in the embryo long before we can see what is to become of them. The best example of this kind is to be found in the early development of fishes formulated in His's brilliant theory of concrescence. According to His, the word "mechanical" is to be applied to the movements of these avalanches of tissues and their influence upon one another in gaining their final position and not to the cause of growth.

One of the characteristics of His's embryological work is that he viewed the embryo as a whole; he always approved of embryological papers which carried a subject to its logical conclusion.¹⁰ As he improved the microtome more and more, so that he could cut serial sections fifty microns thick, he constantly kept in mind the relation of the individual section to the embryo as a whole; this led to his well-known method of graphic reconstruction in 1868. At that time haphazard sections of an embryo were often compared with chance sections of older embryos which led to all kinds of erroneous conclusions. In the course of time, he perfected his method by drawing an enlarged picture of the embryo upon ruled paper into which he projected in their proper positions the sections

enlarged to the same scale. Photography, as well as a new instrument which he invented, the embryograph, aided him in his work. In order to be better able to compare stage with stage, the reconstructions were converted into models which were duplicated by Ziegler; these models now form the most valuable asset of many of the embryological museums the world over. More accurate models can be made by drawing the sections upon wax plates which when placed upon one another reproduce the embryo; this method, invented by Born, was used to a very great extent by His in his later years.

Until this time the embryological campaign had been conducted by His from all sides, but one great fortress, the human embryo, still stood before him. Human embryos were very difficult to obtain and the literature upon the subject was meagre and poor, but His was soon able to select a few important stages from a mass of poor material, normal and pathological. These he studied with such care that the knowledge of the anatomy of the human embryo now exceeds that of any other animal. To be sure the earliest stages were missing, but this mattered but little, for being master of the whole field of anatomy, he was able to produce a model work. Each chapter in his monograph is great and original, giving a mass of information; the work reaches its climax of interest and value in the part on the nervous system.

During the last dozen years of his life, His's attention was taken away from the study of the nervous system by a variety of subjects; thus, for example, he was interested constantly, as his letters show, in the embryology of fishes.¹¹ The work on this latter subject he was able to round out, but it seemed as if his promised work upon the brain would never appear. When I visited him the summer before his death, I found a broken man, working away at a large manuscript which by no means satisfied him. What could be arranged, was published in a monograph upon the brain, which will serve as a foundation for investigation for many years to come. He had hoped to write another volume, but his illness rapidly grew worse, and, unable to work longer, a week before his death, he wrote that his remaining wish was that the end might come soon.

The technical ability of His which had been pretty well trained in the gymnasium, gradually developed farther and proved to be of great value to him as a teacher. Optical instruments of all kinds, magic lanterns and microphotographic apparatus were much used by him; demonstrations with the microscope after each lecture aided in illustration. The pictures which he drew upon the board while lecturing were models of their kind and he developed them before the students in such a manner

that they could be copied gradually while being evolved. The subject matter of his lectures was chosen in a very conservative way, the substance being always sound and free from all kinds of wild theory and speculation.¹²

Early in his career, he had made crude models of the mesentery and the like, for these were subjects the forms of which were difficult to understand. Toward the end of the seventies, modelling was prosecuted on an extensive scale with the aid of the modeller, Steger, resulting in a series of papers on the form and position of the organs which are now standard. His many models have been duplicated and fill an important corner in all anatomical museums; they have made His the founder of a new school of topographical anatomy. The method which His had introduced in embryology were thus also applied to gross anatomy, for he was never satisfied until he could see adult forms in the embryo and the outlines of the embryo in the adult.

In His the power of visualizing forms—the power which enabled him to do his work of reconstruction, to make those wonderful blackboard drawings during his lectures—was developed to an extreme degree. This power was one of his principal gifts, and one of the chief foundations of his achievements in science. As he grew older there was not only an increase in the depth of insight into problems, which is natural in so able a man, but also what is rarer, a very great improvement in the power of expounding his results. His last papers are models, characterized by conciseness of style, great clearness of description and a suppression of all superfluous details.

While His was teaching and investigating, the question of nomenclature came up. Each century, each country, each school, each specialty and each teacher seemed to have a particular group of terms based upon an imaginary normal; the result was that there were so many normals that it was extremely difficult to construct a table of synonyms.

When His and a few others founded the Anatomische Gesellschaft, one of the first questions discussed was the formation of a uniform nomenclature. After much work and expense, His drew up the official report of the international commission, in 1895; it is another standard which the leading teachers and authors have agreed to follow. The new nomenclature is a compromise; it is not radical, but it has reduced the number of anatomical names, including synonyms, by about eighty per cent. It will not be difficult for English-speaking anatomists to accept this terminology, for it differs less from ours than from that of any other language.

If we consider the great amount of work His did upon the form and

position of the organs, upon the general morphology and structure of the brain, upon embryology, histology and histogenesis, and upon anatomical terminology, it may safely be said that there is barely a page in the broad field of anatomy from the ovum to the adult in which his work does not appear.

Early in his career, His showed much interest in physical anthropology as we see by the great monograph he and Rütemeyer published on Swiss skulls. But his time was occupied in so many other directions that it was impossible for him to continue in this kind of work with the inadequate assistance he had at Basel. However, some thirty years later, he had an opportunity to open up a new line of research in anthropology. A skeleton, presumably that of Bach, had been found and His was asked to give an opinion regarding it. It was known that Bach had been buried in an oak coffin near a certain corner of a church-yard. Here among others was found the skeleton of an elderly man (Bach died at the age of sixty-five) in an oak coffin. The skull was found to be peculiar and in it the anatomist could discern the features of the portraits of Bach. His at once proceeded to measure the thicknesses of the soft parts over the bony prominences of the heads of cadavers and found that in average bodies these thicknesses are constant, varying only with age and sex. He next drew averages from the measurements taken from cadavers of elderly men of fair development, and, with these, Seiffner, the sculptor, constructed a clay bust on the skull in question. It was found that the reconstructed bust presented all of the characteristics of Bach even more pronouncedly than do his portraits. The commission that had the matter in charge decided that the skeleton in question was undoubtedly that of Bach. As such, it was reinterred. The musical world through His's studies now has a bust of the great composer.

These results gave His the greatest æsthetic pleasure, for they meant a new victory.¹³ From this time on he was greatly interested in inductive anatomy and when I began my career at the Johns Hopkins, he gave me every possible encouragement in this direction.¹⁴ He often wrote and he often talked about statistical work, but little did I realize the difficulties of it until we began to tabulate a peripheral nervous system from a very large number of individual records. It became apparent from our records that variations are more common in certain parts of the body than in others and this result interested His very much.¹⁵

His was never inclined to develop a school nor was he anxious to have pupils. When I knocked at his door at first I was turned away, but after appearing a number of times, was finally accepted. When he set a problem, it was concisely stated; he outlined the general plan by which

it was to be solved. All of the details were left to the pupil and it annoyed him to be consulted regarding them. He desired that the pupil should have full freedom to work out his own solution and aided him mainly through severe criticism. Specimens and drawings of them which were not analyzed did not appeal to him and he objected much to pictures which appeared to represent a mass of "baked tissue." Through reactions, either with coloring matter or with some destructive reagents, or by means of reconstructions, tissues must be emphasized and we see this characteristic in the illustrations of all of his publications. A His drawing can always be recognized even if it appears without his name attached to it.

His was unwilling to give his own problems to pupils and though later in life he advocated the establishment of research institutes, it is not altogether clear how he reconciled the one attitude with the other. But the institute is rather for routine research and for a discussion of work by leading investigators who consequently formulate problems to be solved by organized united effort; it is not intended to dwarf individual effort in any respect. His was unwilling to write papers for his pupils and the manuscripts they placed before him were improved only through erasure, for he excluded all doubtful evidence and irrelevant matter. "Your paper will be read by a few specialists, and they do not want a treatise on the science," he would say, and this criticism coming to a pupil during the same years that silence on such subjects and encouragement came from Ludwig, proved to be of the greatest value.

His did not have much power to extend his own private work through his assistants and pupils; they were always given the greatest freedom for it was against his nature to enslave them to the least degree. Nor did he possess the patience of a Liebig or a Ludwig in training others to follow in his path. Further, he did not find that successful research can often be stimulated by example, although men, and scientists too, are imitative. He often told me that the acceptance of a discovery is frequently postponed by numerous "confirmatory" publications which are filled with so much crude and irrelevant matter that the real point is buried again; and that the desire of authors to attract attention often induces them to invent names and write much, thereby making the answer to a question more obscure than it was before.

Several visits to the Zoological Station at Naples made a profound impression upon His for it showed him what organization can do for research, and in 1886 he published a paper on the necessity of research institutes. In this he pointed out that the function of such institutes is (1) to solve problems which exceed the power of one man to settle,

and (2) to collect, classify and conserve all the material relating to such problems. After a whole life-time has been occupied in collecting material, it seems a great pity that so much work should be lost at the death of the collector; could such material and such lives be made available for the solution of the great problems by an institute or institute of institutes (as is the case in astronomy) more progress might be made. In the foundation of such an institute the many details of embryology and neurology should first be surveyed; were the field divided, this could be done quickly. Problems would have to be formulated, and a common nomenclature and standard of measurements agreed upon. In no case should individual effort be hampered. Conferences would be necessary from time to time to compare, to criticise results and to formulate new problems and new plans for their solution, thus aiding all with the best, as should be the case in ideal scientific investigation. The underlying thought is to extend the power of able investigators through the whole science without dwarfing in any way individual effort; in this way the best qualities of all could be made to serve the progress of science.¹⁶ The Anatomische Gesellschaft was founded with this as one of its objects in view; it naturally resulted as we have seen in the casting of a uniform nomenclature. Two great steps had thus been taken and His lived to see the beginning of a third.

The tendency in the world during all of His's life was more and more towards specialization and organization in science, a movement which gradually became international. An index of this tendency may be seen in the organization of the International Association of Academics and through its machinery, His was able to launch his favorite scheme.

Unlike many promoters of science, His was not an impresario and consequently the learned world had full faith in him. What he advocated always was the advancement of science. The possibility of personal gain was excluded from his thoughts as is shown by his attitude towards endowments, especially the Nobel Fund.¹⁷ He maintained all along that a research fund must fall into the right hands if it is to benefit science and that it was of positive injury to science when in the hands of impressarios.¹⁸

His proposed at the first meeting of the International Association of Academics, held in Paris in 1901, that a commission be appointed for the promotion of the study of human embryology and another for the study of the anatomy of the brain. The Association agreed to the appointment of the latter, at the same time recommending that for the present, human embryology should be taken up by the anatomical societies. Later, upon the recommendation of the Saxon Academy, the Royal

Society of London appointed a Neurological Commission of seven members with His as chairman. The Commission had been established and three bulletins had been published preparatory to the first general meeting, but His died before the meeting convened. His plan will live, for it has taken deep root and has the approval of leading anatomists of the world.¹⁹

The life of His was a life of work, and his energy, industry and endurance were so great that he hardly knew the meaning of leisure. He possessed the qualities of a courageous leader, but lacked the magnetism that compels many admirers and followers. He was a daring and original investigator, possessing great technical ability and artistic feeling; he was fearless and honorable in controversy and knew no compromise. He was a great character, true to his family, true to his friends and true to science.²⁰

Through His, another milestone has been set for anatomy. Through him the great mother science has given birth to a new science, histogenesis. His career is marked by a monument of neurological research which is unique. His's life was that of the ideal scholar. During youth he was strengthened through his own efforts, directed by great masters. During middle age, he won many victories for anatomy, improving the science in all its parts. In old age, he completed and rounded up his work, leaving a great legacy to his survivors, no small part of which consists of wise plans for future work.

REFERENCES AND EXTRACTS FROM LETTERS.

¹ Wilhelm His was born in Basel on July 9, 1831. His father, Edward His, was a son of the Swiss statesman, Peter Ochs. In 1818, when Edward Ochs became engaged to be married to Anna La Roche, he assumed the name of His, the maiden name of his father's mother. He did this with the consent of Peter Ochs in order to remove the ridicule of his name from which he had undoubtedly suffered. After graduating from the Gymnasium, His studied medicine in Basel, Bern, Berlin, Würzburg, Prague and Vienna, returning to Basel to take his doctor's degree, in 1854. Later, he studied in Paris, returning to Basel as "Privatdocent" in 1856. In the summer of 1857 he was again in Berlin and in the autumn he was appointed Professor of Anatomy and Physiology in Basel. In 1872 he accepted the call to the chair of anatomy at Leipzig, where he died May 1, 1904.

A charming account of His's early life is given by him in his *Lebenserinnerungen (als Manuskript gedruckt)*, Leipzig, December, 1903. See also W. SPALTEHOLZ, Zum siebzigjährigen Geburtstag von Wilhelm His, *Münchener Medizinischen Wochenschrift*, No. 28, 1901; and *Wilhelm His*, *ibid*, No. 22, 1904. RUDOLPH FICK, *Wilhelm His, Anatomischer Anzeiger*, Vol. 26, 1904. B. RAWITZ, *W. His, Naturwissenschaftliche Rundschau*, No. 24, 1904.

FRANCIS DIXON, *Prof. Wilhelm His, Journal of Anatomy and Physiology, Vol. 38, 1904.* W. WALDEYER, *Wilhelm His, Sein Leben und Wirken, Deutsche Medizinische Wochenschrift, Nos. 39, 40 and 41, 1904.* J. KOLLMANN, *Wilhelm His, Worte der Erinnerung. Verhandl. der Natur. Gesell. in Basel, Bd. 15, 1904.* J. MARCHAND, *Wilhelm His, Nekrolog, Bericht. d. K. s. Gesell. d. Wiss., Nov. 14, 1904.*

² Ich hatte in Berlin bei Johannes Müller und bei Remak tiefe Anregungen erfahren, im übrigen aber einen nur mäszigen Schatz von geordneten Kenntnissen eingespeichert. Es hatte mir an sicherer Führung gefehlt: unter den Medizinern hatte ich keine Gleichgesinnten gefunden, und mein eigentlicher Freundeskreis bestand aus Landsleuten, meistens Theologen und Juristen. Schöne Gelegenheiten, mir gründlichere physikalische und chemische Kenntnisse zu erwerben, habe ich verpasst und statt dessen einige recht sterile medizinische Vorlesungen abgesessen. Auch die historischen Vorlesungen von Ranke und die geographischen von Ritter, von denen meine Freunde soviel Interessantes zu erzählen wussten, hätte ich ohne Opfer an Fachbildung besuchen können.—*Lebenserinnerungen*, p. 28.

³ An jene Zeit absoluter Arbeitsfreiheit habe ich seitdem oft mit Sehnsucht zurückgedacht. Allerdings bot sie auch die Gefahren des Sichverlierens, so habe ich einmal acht Tage lang an der Herstellung eines Glasbläsartisches gezimmert, bin aber dann, nach dessen notdürftiger Vollendung, von einem gehörigen Katzenjammer über die sinnlos vergeudete Zeit heimgesucht worden. Im Grund habe ich aber in späteren Jahren die Erfahrung gemacht, dass für den Fortgang eigener geistiger Arbeit die Belastung mit einem mäszigen Pflichtenpensum vorteilhafter ist als die absolute Freiheit, und insbesondere habe ich oftmals beim Beginn ersehnter Ferien gefunden, dass zugleich mit dem Eintritt freier Zeitverfügung eine Erschlaffung der geistigen Spannkraft sich einstellte, die erst allmählich und durch Zwang sich wieder überwinden liess. Das Gefährlichste ist hierbei das Abwartenwollen von Arbeitsstimmungen; solche wirklich fruchtbare Stimmungen können ja zeitweise unverhofft einbrechen, viel häufiger aber sind sie nur dadurch erreichbar, dass man sich erst gewaltsam durch öde und anscheinend unfruchtbare Anfänge hindurch kämpft. Hat man einmal sein Arbeitsziel klar vor Augen, dann lernt man auch bald die kleinsten Zeitatfälle des sonstigen Tagewerkes ergiebig zunutze zu ziehen.—*Lebenserinnerungen*, p. 48.

⁴ Die meisten jungen Männer müssen nach Abschluss ihrer Universitätszeit eine Periode des Missbehagens durchmachen, bis es ihnen gelungen ist ihre idealen Bestrebungen in eine Thätigkeit für's Leben umzusetzen.—*From a letter of March 20, 1887.*

⁵ Es ist ein schweres, dem seiner Natur getreu bleibenden Forscher auferlegtes Geständniss, dass die letzten Ziele, für deren Verfolgung er seine ganze Kraft einsetzt, hier, wie auf allen Gebieten der Forschung, in um so entlegener Ferne rücken, je weiter er auf dem in ihrer Richtung führenden Wege voranschreitet. In der kräftigenden Arbeit selbst, im Bewusstsein sicherer Voranschreitens und in den reichen, am Wege ihn erwartenden Früchten findet er den vollen Ersatz für alle geübte Entzagung.—*Unsere Körperform, 1874*, p. 215.

⁶ Ist es ja doch die Gabe geistvoller Naturen, dass sie, auch bei beschränkten Hülfsmitteln materieller Erkenntniss, Beziehungen zuahnen und in ihrem

Zusammenhang zu durchschauen vermögen, die Anderen bei weit reicherem Material nur stückweise zugänglich sind, und dass sie selbst im Irrthum oft Gesichtspunkte eröffnen, die der langsam und mühselig vordringenden Einzelnforschung als Wegweiser für die Richtung ihres Ganges dienen können.—*Die Häute und Höhlen des Körpers* (1865), *Archiv für Anatomie*, 1903, p. 369.

⁷ Soll ich zum Schluss noch einmal versuchen, die histologischen Rollen der Keimschichten zu sondern, so komme ich zu folgender Aufstellung:

Der Epiblast liefert das Nervengewebe und die Horngewebe.

Der Hypoblast gliedert sich in

den embryonalen Mesoblast, die gemeinsame Anlage für das quergestreifte und glatte Muskelgewebe, für die Epithelien des Genitalapparates und für die embryonalen Bindesubstanzen.

das ausserembryonale Mesenchym,

den Angioblast, die Anlage des Blutes und der Blutcapillaren,

das Endoderm, die Anlage der Epithelien und Drüsen des Eingeweide-rohres.

Der Lecithoblast, da, wo er zur Entwicklung kommt, bildet einen Theil des Hypoblast.

Das alte Räthsel erweist sich zur Zeit immer noch ungelöst: noch können wir nicht sagen, weshalb ein Theil der gegebenen Anlagen zu Bindesubstanzen wird, und was die Blut- und Capillarzellen bestimmt, so frühzeitig und so scharf sich von ihren scheinbar so nahen Verwandten, den Zellen der Bindesubstanzen, zu scheiden.—*Lecithoblast und Angioblast der Wirbelthiere*, Abhandl. d. K. Säch. Gesellschaft. d. Wiss., Bd. 26, 1900, p. 326.

⁸ Ich schliesse diesen in jeder Hinsicht fragmentarischen Aufsatz über die intramedullaren Faserbahnen des Gehirns mit der Bemerkung, dass er zur Zeit nicht viel mehr zu bieten vermag, als ein Arbeitsprogram für kommende detailliertere Forschungen. Noch sind wir eben in Erkenntniss dieser Dinge in den allerersten Anfängen, und es bedarf hier, wie anderwärts, zäher Arbeit bis die Entwicklungsgeschichte des Gehirns nach ihren verschiedenen Richtungen hin befriedigend kann klar gelegt werden. Zur Zeit kann ich nur angeben, wo diese Arbeit einzusetzen hat. Früher oder später wird man auf diesem Gebiet zum System organisierter gemeinsamer Arbeit überzugehen haben.—*Die Entwicklung des Menschlichen Gehirns*, Leipzig, 1904, p. 175.

⁹ Im Leben unsrer Universitäten macht sich bei aller anscheinenden Fortdauer ihrer Leistungen, und auch bei ununterbrochenem Ersatz abgehender Kräfte durch neu eintretende, eine ganz bestimmte Periodicität der Entwicklung geltend. Für die Gesamtuniversität und für die Facultäten folgen auf Perioden geistigen Aufschwunges solche der Ruhe und des Rückgangs. Aeussere und innere Bedingungen wirken dabei zusammen und es ist nicht immer leicht, deren Ineinandergreifen zu verstehen. Eine Grundbedingung muss aber stets erfüllt sein, falls eine Körperschaft blühen soll. Die Körperschaft muss kräftige und zielbewusste Führer besitzen, welche deren Geist in bestimmte Bahnen zu lenken und unter ihren Gliedern die Gemeinsamkeit des Strebens zu sichern wissen.

Solch ein führender Geist ist in unsrer Facultät während mancher Jahrzehnte Ernst Heinrich Weber gewesen, welcher vom Jahr 1821 ab die Pro-

fessor der Anatomie und späterhin (von 1841 ab) noch die der Physiologie bekleidet hat. Die Spuren seiner mächtigen Persönlichkeit haben sich als bleibende erhalten nicht nur in den Acten unserer Facultät, sondern noch tiefer begründet in denen der Wissenschaften, die er vertreten und die er um ausgedehnte neue Gebiete bereichert hat.

Bis zum Jahre 1865 hat Ernst Heinrich Weber, von seinem Bruder Eduard unterstützt, die Doppelast der beiden ausgedehnten Fächer getragen. Dann aber, als die Neuschöpfung einer physiologischen Anstalt in Aussicht genommen wurde, und dadurch neue Verpflichtungen an den Lehrer der Physiologie herantreten sollten, zog sich der alternde Gelehrte auf seine ursprüngliche Anatomieprofessur zurück, und es ist nun auf Ostern 1865 (unter dem Dekanat Wunderlichs) die Berufung von Karl Ludwig als Professor der Physiologie und Director des neu zu begründenden physiologischen Instituts erfolgt.

Die Initiative zu diesen Neuerungen ist von der königlichen Regierung ausgegangen. Im Sinn ihres hohen Monarchen, des Königs Johann, hatten sich die einsichtigen Leiter des Ministeriums, Hr. Staatsminister v. Falkenstein und Hr. Geh. Rath Dr. Hübel, die Aufgabe gestellt, die Universität Leipzig mit allen aufwendbaren Mitteln zu neuem Glanze zu erheben. Die physiologische Anstalt wurde als das erste Glied einer Reihe von Neuschöpfungen geplant, deren Endziel die Umgestaltung des gesammten naturwissenschaftlichen und medizinischen Unterrichts sein sollte. In der Wahl von Professor Ludwig hat die k. Regierung eine besonders glückliche Hand bewiesen, denn sie gewann an ihm für ihre ferneren Entscheidungen einen vermöge seiner Einsicht und seiner organisatorischen Kraft ganz besonders befähigten Rathgeber. Ludwig's Einfluss hat sich während der v. Falkenstein'schen Periode weit über das medizinische Facultätsgebiet hinaus erstreckt, und seiner Anregung sind von den bedeutendsten Berufungen jener Zeit zu verdanken gewesen. Später, nachdem einmal die Organisation naturwissenschaftlichen Unterrichts für Leipzig erreicht und nachdem auch das Cultusministerium in andere Hände übergegangen war, hat sich Ludwig auf sein engeres Arbeitsgebiet zurückgezogen. Was er aber auf diesem Gebiete geleistet hat, das hat den Ruhm der Leipziger Universität bald durch alle Länder verbreitet.—KARL LUDWIG und KARL THIERSCH, *Beilage, Allgemeine Zeitung*, Nr. 164. 19 Juli, München, 1895.

¹⁰ Ich danke Ihnen für den inhaltsreichen Aufsatz über die Darmentwicklung, die in der mir überreichten Jubiläumsschrift thatreich hervortritt. Alle diese Bezeugungen haben mich herzlich gefreut. Dauernd wird die Befriedigung über Ihre Arbeit sein, die ein bis jetzt so wenig klarer Gebiet endgültig in's Reine bringt. Was ja bei den meisten unserer bishörigen entwicklungsgeschichtlichen Vorstellungen fehlt, das ist die Beobachtungsgrundlage für die Uebergangsphasen aus den früh embryonalen in die foetalen und von da in die ausgebildeten Stufen. Für den Darm haben Sie nunmehr die ganze Kette vom Anfang bis zum Ende zusammengefügt und das halte ich für einen grossen Fortschritt.—From a letter of October 29, 1897.

¹¹ So weit ich über solche freie Augenblicke verfüge, widme ich sie noch meiner alten unglücklichen Liebe den Knochenfischen. Ich habe seit dreissig Jahren schon unendlich viel Zeit damit verloren, sie sind ein methodisch

sehr schwer zu bearbeitendes und launisches Material, und doch locken mich die unüberwundenen Schwierigkeiten und öffnen Fragen immer wieder zu neuen Anlaufen.—*From a letter of December 25, 1898.*

¹² Wie bei der wissenschaftlichen Arbeit, so tritt auch bei unserer heutigen Lehrweise der Respect vor der Thatsache in den Vordergrund, und wir bemühen uns in erster Linie auch unsere Schüler dazu zu erziehen. Beim naturwissenschaftlichen und somit auch beim medizinischen Unterricht ist unsere Sorge, dem Anfänger die Kunst unbefangener Beobachtung beizubringen. Wir halten ihn an, die Sinneswahrnehmungen scharf zu trennen von den daran sich anknüpfenden Schlussfolgerungen, wir warnen ihn vor der Beeinflussung durch vorgefasste Meinungen und belehren ihn über die Täuschungsquellen, die in unsren eigenen Sinnen sowie in unsren besten Apparaten enthalten sind. Vor allem aber suchen wir den Schüler dazu zu bringen, dass er sich angewöhnt, das Gebiet eigener Erfahrungen selbständig zu klaren Begriffen zu verarbeiten. So klein Anfangs das Capital an solch eigenem Erwerb sein mag, so gewährt es dem Besitzer doch bald das Gefühl einer bestimmten geistigen Freiheit und Unabhängigkeit, das Gefühl des tüchtigen Menschen.

Was hat nun aber diese, vorwiegend auf Schärfung der Kritik hinstrebende Form der Schulung mit der Spaltung der Lehrfächer zu thun? Der Zusammenhang ist leicht nachzuweisen. So lange es sich um blosse Ueberlieferung systematisch geordneter Begriffe in dogmatischer Form handelt, ist ein fleissiger Gelehrter mit Hilfe der nöthigen Lehrbücher, der duces Arnemann, Gaubius und Metzgerus im Stande, ein ausgedehntes Gebiet als Lehrer zu umspannen, ja selbst vom Ueberspringen von einem Fache auf ein anderes, mehr oder minder entlegenes, wird ihn kein inneres Hinderniss abhalten. Wenn wir hören, dass in einem früheren Jahrhundert die Lehrfächer innerhalb der philosophischen Facultät jedes Jahr frisch ausgelöst wurden und dass auch nach Beseitigung dieses Modus noch die Verpflichtung bestand, dass ein jedes Facultätsmitglied allen Fächern gerecht sein musste, so ist diese heutzutage undenkbare Einrichtung dadurch verständlich, dass in jenen Perioden die Bedeutung der allgemeinen Gelehrtenbildung über diejenige der Fachbildung weit überwog, während wir nunmehr auf dem entgegengesetzten Standpunkt stehen. Sowie verlangt wird, dass der Lehrer die wissenschaftlichen Ergebnisse seiner Disciplin anstatt blos in dogmatischer Form, auch nach ihrer Begründung dem Schüler mittheile, so fällt eine Hauptseite des Unterrichts in die wissenschaftliche Methodik.—*Ueber Entwickelungsverhältnisse des Akademischen Unterrichts, Rektoratsrede, Leipzig, October 31, 1882, p. 33.*

¹³ Ludwig's Forscherwaffen waren eine ungemain scharfe Analyse der ihm vorliegenden Naturerscheinungen, eine stets klare Fragestellung und eine absolute Sicherheit seiner Methodik. Dabei verfügte er aber auch über eine ausreichende Dosis jenes Findersinnes, ohne den in Erforschung der lebenden Natur selbst die klarsten Denker oft machtlos bleiben. Die Natur lässt sich nicht immer mit Logik zwingen, ihre Wege sind nicht selten versteckt, und sie enthüllen sich nur dem, der sich in ausdauernder und treuer Beobachtung den Blick auch für deren unscheinbare Spuren geschärft hat. Die unmittelbare Liebe zur sinnlichen Beobachtung hat aber Ludwig im hohen Maasse

besessen, und für ihn ist ein gelungenes Präparat oder ein schlagender Versuch stets Gegenstand eigentlich ästhetischen Genusses gewesen.—CARL LUDWIG, *Gedächtnissrede, Bericht, d. K. s. Gesell. d. Wiss., November 14, 1895*, p. 6.

¹⁴ Ihre Bestrebungen eine inductive anatomische Unterrichtsmethode zu schaffen, interessiren mich sehr lebhaft. Wenn es Ihnen mit fünfzig Schülern gelingt, zum Ziel zu kommen, so ist dies jedenfalls eine anerkennungswerte Leistung. Vor Kurzem publicirte der bekannte, Dr. Schweninger, einige Aufsätze über die Erziehung von Medizinern, worin er überhaupt das Präpariren verwarf und meinte, man soll die Anatomie gleich am Lebenden vornehmen, die Studenten durch Percussion, u. s. w. die Organe auf den Körper zeichnen lassen, u. s. w. Unsere Medizinererziehung ist zwar krank an zu vielem Auswendiglernen von Bücherweisheit und gewiss könnte auch in der Anatomie dem Studenten manches osteologisches Detail erlassen werden. Aber abgesehen davon, ist ja der Präpariersaal eine so wichtige Schule der Beobachtung und der Handfertigkeit, dass eine grosse Beschränktheit dazu gehört, das anatomische Präpariren beseitigen zu wollen.—*From a letter of December 31, 1896.*

¹⁵ His's influence in America has been great, greater than in any other country, even Germany. He took a lively interest in our whole development, in the development of our universities, scientific societies and journals. He was much pleased with the numbers of the American Journal of Anatomy, and appreciated above all the leading article by Bardeen and Lewis. "Auch darüber habe ich mich gefreut dass Sie mit so viele Andere zusammen arbeiten." He always approved of coöperation.

¹⁶ Was Sie mir damals von "Carnegie Institution," geschrieben haben, muss uns, diessseits des atlantischen Oceans Lebende mit innigem Neid erfüllen. Es ist indessen keine Frage, wir sind in eine Periode eingetreten, in der die zu leistende Arbeitssumme immer grösser und die Ansprüche an Reichlichkeit des Materials und die Präcision seiner Durcharbeitung immer strenger werden und da hilft eben schliesslich nur ein wissenschaftlicher Grossbetrieb mit guter Organisation. Noch haben wir in Deutschland bei aller Arbeit ein zu planloses Durcheinanderrogen, und zu viel Kraft geht in persönlicher Reibung verloren. Der Ehrgeiz ist ein wichtiger Antrieb zur Arbeit, aber anderseits führt er auch vielfach dahin, dass die Arbeiter anstatt sich zu unterstützen, sich gegenseitig herabzumindern suchen. . . . Noch vor zehn Jahren hatte mir die Organisation eines grosseren rein wissenschaftlichen Institutes, die grösste Freude gemacht. Mit zwei und siebenzig Jahren weiss man aber, dass die Arbeitszeit nur noch knapp zugemessen ist, ganz abgesehen davon, dass die Arbeit viel langsamer von der Hand geht.—*From a letter of March 17, 1903.*

¹⁷ Ich hatte im vorigen Sommer einen Anlauf genommen, um die Begründung besonderer entwickelungsgeschichtlichen Institute und Gehirnstitute in Anregung zu bringen, aber bis jetzt habe ich noch nicht Viel erreicht. Es fehlen uns in Deutschland und in Europa jene Milliardäre die bei Ihnen so fix bei der Hand sind, wenn grosse Schöpfungen fundirt werden sollen. Das immense Capital, dass der Ingenieur Nobel für wissenschaftlichen Zwecke vermacht hat, ist dadurch nutzlos, dass er die Vertheilung der Zinsen in Form von Preisen

bestimmt hat. Da diese Preise jedes Jahr vertheilt werden sollen so wird, wie ich fürchte, die Zutheilung bald zu Parteisache werden und viel Unfrieden herbei führen.—*From a letter of December 31, 1902.*

“Wird durch solche Preise die wissenschaftliche Arbeit wirklich gefördert?” Ich glaube, man kann diese Frage ruhig verneinen: kein aus innerem Antrieb arbeitender Forscher wird dadurch, dass ihm das Schicksal eine grössere Summe Geldes in den Schooss wirft, ein Anderer werden. Er wird eben über die Ehre des Preises und über den empfangenen Betrag sich freuen, im Uebrigen aber seinen Gang weiter gehen, als ob Nichts geschehen wäre. Und wer den Preis nicht bekommt, wird nicht anders verfahren. Höchstens liegt für den letzteren, wenn er nicht edel veranlagt ist, die Versuchung vor, dem begünstigten Collegen, oder denen, die über den Preis zu bestimmen hatten, unfreundliche Gefühle nachzutragen.—*Ueber wissenschaftliche Stiftungen, Bericht. d. K. s. Gesell. d. Wiss.*, 1901, p. 434.

¹⁸ Sie haben an ihren neueren amerikanischen Universitäten einen kräftigen Nervus rerum, und wenn reiche Hilfsmittel in die richtigen Bahnen kommen, so lässt sich ja Vieles in verhältnismässig kurzer Zeit erreichen. Die Haupt-sache bleibt immer dass die Führung solch fortschreitender Bewegungen in den Händen von Männern bleibt, die wissen, woraus es bei geistigen Schöpfungen ankommt. Es ist immer befriedigender, völlig Neues zu schaffen, als am Alten herumzuflicken. Letzters Schicksal fällt uns in Europa nun allzu oft zu. Augenblicklich soll wieder an unsern Examenreglementen geflickt werden, eine Arbeit die nur wenig Freude bringt, da der Ballast alter Vorurtheile und Wiederstände nicht über Bord geworfen werden kann.—*From a letter of April 22, 1899.*

¹⁹ GENERAL OUTLINE OF THE DEVELOPMENT OF HIS'S INSTITUTE FOR THE STUDY OF THE BRAIN.

A.—*Proposition to the International Association of Academies, Paris, April 20, 1901.*

Die Internationale Association der Akademien möge eine Fachcommission aufstellen zur Berathung der Mittel und Wege, wie auf den Gebieten, eines-theils der menschlichen und thierischen Entwicklungsgeschichte, anderntheils der Hirnanatomie eine nach einheitlichen Grundsätzen erfolgende Sammlung, Verarbeitung und allgemeine Nutzbarmachung von sicherem Beobachtungs-material erreicht werden kann.

B.—*Decision of the Association of Academies.*

1. Die Berathung der auf menschliche und thierische Entwicklungsgeschichte bezüglichen Abschnitte des Antrages ist vorerst den betreffenden Fachvereinen (den anatomischen Gesellschaften) zu überlassen.

2. Dagegen setzt die Internationale Association der Akademien eine Special-commission nieder, die eine nach einheitlichen Grundsätzen erfolgende Durch-forschung, Sammlung und allgemeine Nutzbarmachung des auf Gehirnanatomi-e bezüglichen Materiales zu berathen hat. Die Commission hat insbe sondere die Schaffung eines internationalen Systemes von Centralinstituten in Erwägung zu ziehen, in denen die Methoden der Forschung entwickelt, das vorhandene Beobachtungsmaterial aufgespeichert und der allgemeinen Benutzung der dabei interessirten Gelehrten zugänglich gemacht werden.—*An-*

trag der Kön. Sach. Ges. an die Royal Society of London, Ber. d. K. s. Ges. d. Wiss., February, 1902.

C.—*Objects of the Institute.*

1. Die Aufspeicherung und Zugänglichmachung von wissenschaftlichem (normalem) Material an Präparaten, Modellen, Photogrammen, Zeichnungen u. s. w.
2. Die technische Hilfeleistung bei wissenschaftlichen Untersuchungen.
3. Die Aufbewahrung von wertvollem experimentellphysiologischem und pathologischem, bereits bearbeitetem oder noch zu bearbeitendem Material.
4. Die Bewältigung grösserer, über die Krafte einzelner hinausgehender Aufgaben, soweit solche zur Kooperation sich eignen.—*Antrag der von der Internationalen Association der Akademien Niedergesetzter Commission für Hirnforschung der Generalversammlung der Association in London zum 25 Mai, 1904, vorgelegt, Leipzig, 1904.*

D.—*Organisation of the Institute.*

1. Arbeitsfeld und Arbeitsweise bleiben jedem einzelnen Institute überlassen. Es sollen jedoch angestrebt werden:
 - a. Eine einheitliche Nomenklatur.
 - b. Verwendung eines einheitlichen Masses und Gewichtes.
2. Alljährlich statthen die Institute der Centralkommission einen Bericht über ihre Thätigkeit ab. Dabei sollen der Bestand und die Zugänge an Druckwerken, Abbildungen, Modellen und Präparaten mitgetheilt werden.
3. Die Institute sind gehalten ihre Arbeitsmaterialien und die Sammlungen ihrer Präparate einander unter sich, sowie den derselben bedürftigen Forschern nach Möglichkeit zugänglich zu machen.—*Bericht, etc., Bericht. d. K. s. Gesell. d. Wiss., June 8, 1903.*

D.—*Sections.*

1. Die systematische Anatomie des menschlichen Centralnervensystems, einschlieszlich der Anthropologie.
2. Die vergleichende Anatomie.
3. Die histologische Forschung.
4. Die entwickelungsgeschichtliche Forschung.
5. Die Physiologie, einschliesslich der physiologischen Psychologie.
6. Die pathologische Anatomie, experimentelle Pathologie und Teratologie.
7. Die klinische Forschung.—*Entwurf. Motiv zu den Anträgen, etc., Leipzig, January 3, 1904.*

F.—*Special Committees.*

1. Waldeyer, Cunningham, Mall, Manouvrier, Zuckerkandl.
2. Ehlers, Edinger, Giard, Guldberg, Elliot Smith.
3. Golgi, Ramon y Cajal, Dogiel, van Gehuchten, Retzius.
4. His, Bechterew, v. Kölliker, v. Lenhossek, Minot.
5. H. Munk, Horsley, Luciani, Mosso, Sherrington.
6. Obersteiner, Dejerine, Monakow, Langley, Weigert.
7. Flechsig, Hentschen, Ferrier, Lannelongue, Reymond.—*Protokoll von der Internationalen Association der Akademien Niedergesetzten Centralkommision für Gehirnforschung, January 11, 1904. Bericht. d. K. S. Gesell. d. Wiss., 1904.*

²⁰ Es that mir wohl aus Ihrem Brief, wie aus vielen Andern, die ich bekommen habe, zu sehen, wie mein Mann nicht nur durch seine Wissenschaft, sondern noch mehr durch sein Leben und seinen Karakter seinen Schülern etwas Gutes erwiesen hat.—*From a letter from Frau Professor His of June 8, 1904.*

Ich bin mit diesen Aufzeichnung an einen Punkte angelangt, wo ich sie abschliessen kann. In reichem Wechsel sind mir beim Niederschreiben obiger Blätter Bilder vor Augen getreten von einer Fülle von trefflichen und von hervorragenden Menschen, mit denen ich im Laufe meiner Entwicklungsjahre in Beziehung getreten bin. Gar manche Namen hätte ich der Schar noch beifügen können. Von allen diesen Menschen habe ich gelernt oder sonstwie Gutes empfangen. Die weit überwiegende Mehrzahl derselben sind längst dahingeschieden, allen aber bewahre ich ein dankbares Andenken. Mögen andere dereinst auch von mir dasselbe sagen können.—*Lebenserinnerungen.*

THE DEVELOPMENT OF THE THORACIC VERTEBRÆ IN MAN.

BY

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WITH 7 PLATES.

There is a somewhat extensive literature dealing with the development of the spinal column in various vertebrates. The chief stages in its differentiation are fairly well determined. Special attention has been given to the early development in the lower vertebrates. The recent literature on this subject up to 1897 has been reviewed by Gaupp, 97.¹ Somewhat less attention has been devoted to the mammals. To Froriegp, 86, is due a valuable account of the development of the cervical vertebrae in the cow, and to Weiss, 91, an important description of the development of the thoracic and cervical vertebrae in the white rat.

We shall not attempt to enter here into a description of the early stages of differentiation in the spinal axis; that is of the period covering the formation of the chorda dorsalis and of axial and peripheral mesoblast, the differentiation of primitive segments, and the origin of the axial mesenchyme. This period in the human embryo has been well treated by Kollmann, 91, and some account of it has previously been given in this JOURNAL (Bardeen and Lewis, 91). We shall therefore proceed at once to a consideration of vertebral differentiation in the axial mesenchyme.

Vertebral development in the embryo may be divided into three overlapping periods: a membranous or blastemal, a chondrogenous, and an osseogenous.²

¹ Among more recent papers may be mentioned, those of Baldus, 91; Hay, 97; Kapelkin, 90; Männer, 99; Männich, 92; Ridewood, 91; and Schauinsland, 93.

² In the text books the first of these periods is usually called the *precartilage*, *prochondral*, or *Vorknorpel* stage, but the condensed tissue from which the skeletal parts are derived gives rise not only to cartilage but also to perichondrium and to ligaments. Recognizing this fact, Schomburg, 90, has

THE BLASTEMAL PERIOD.

The division of the axial mesenchyme into segments, sclerotomes, which correspond to the myotomes and spinal ganglia, is marked at an early stage by intersegmental arteries. Schultze, 96, has shown that the segmental differentiation of the axial mesenchyme extends into the region dorsal to the spinal cord. Ventrally it does not, however, extend quite to the chorda dorsalis. Fig. 1, Plate I, illustrates the conditions existing in the thoracic region of man at this period.

v. Ebner, 88, found in the embryos of several vertebrates a fissure which divides each sclerotome into an anterior and a posterior portion. Schultze, in 1896, showed, that in selachians and reptiles this fissure is represented from the time of its formation by a diverticulum which communicates with the myocoel. In birds the diverticulum arises secondarily and later becomes fused with the myocoel, and in mammals it arises after the myocoel has disappeared.

In man the fissure becomes distinct in the thoracic region at about the end of the third week of development (Fig. 2, Plate 1).³ At this period the median surface of each myotome has become converted into muscle fibres (Fig. 2, *Myo.*). At the same time the mesenchyme in the postero-lateral region of each sclerotome has become condensed so that it appears, in a stained section, dark when compared with that of the anterior half (Fig. 2). At the lateral margin of the anterior halves of the sclerotomes the spinal nerves extend out toward the thoracic wall (Fig. 2, *Sp. N.*). The division between the sclerotomes is still marked by the intersegmental arteries (Fig. 2). About the chorda dorsalis the cells of the axial mesenchyme become densely grouped into a perichordal sheath. The long axes of the cells lie parallel with the chorda (Fig. 2, *Pch. S.*).

The condensation of tissue which distinguishes the posterior sclerotome half begins, as mentioned above, in the posterior lateral area of each

called the condensed-tissue stage, the mesenchymal period, and restricted the term Vorknorpel to the earlier stages of the formation of cartilage. The term "blastemal" is now-a-days commonly used to designate a mass of mesenchymal tissue from which organs are to be differentiated, and is applied to the tissue of the limb-bud before differentiation has commenced. It seems to me that it would be well to extend this term to the structures first differentiated in the limb. Thus, "scleroblastema" would mean the tissue differentiated from the blastema of the leg and destined to give rise to skeletal structures; myoblastema, the tissue differentiated for the muscles, and dermoblastema that destined for the skin.

³ The figures on this and the following plates are based upon embryos belonging to the collection of Prof. Mall. I am greatly indebted to him for the use of these embryos.

sclerotome. From here the condensation extends dorsally between the medial surface of the posterior half of the corresponding myotome and spinal ganglion and gives rise to a dorsal, or neural, process (Figs. 5 and 6, Plate 2, *N. Pr.*). At the same time it proceeds ventrally along the distal margin of the corresponding myotome and gives rise to a ventral or costal process (Figs. 5 and 6, *C. Pr.*) ; and medially toward the chorda dorsalis, giving rise to a process which joins about the chorda dorsalis with a similar one, from the other side of the segment (Figs. 5 and 6, *Disk*). These median processes by their fusion form what has been termed by Weiss, or, a "horizontal plate." "Primitive disk" seems to me perhaps a better term.

The whole mass of condensed tissue which gives rise to the primitive dorsal, ventral and median processes has received various designations, of which that given by Froriep, 83, "primitive vertebral arch" seems to be the most widely accepted. Since, however, it represents much more than a vertebral semi-arch, I have previously, 99, suggested for it the term "scleromere."⁴

Figs. 8, 9 and 10, Plate III, represent wax-plate reconstructions of several scleromeres from the thoracic region of Embryo II, length 7 mm. The outlines of the condensed tissue are not so sharp in nature as it is necessary to make them in a model of this kind. It is believed, however, that the general form relations are here fairly accurately shown. In Fig. A, Plate II, of the article by Bardeen and Lewis are shown the relations of the scleromeres to other structures.

During the period of differentiation of the scleromeres the myotomes undergo a rapid development. The median surface of each myotome gradually protrudes opposite the fissure of v. Ebner. The dorsal and ventral processes of each scleromere are then slowly forced into the interval between the belly of the myotome to which it belongs and the one next posterior, and thus finally they come to occupy an intersegmental position. It is not, however, correct to call the early processes of the scleromeres "myosepta," as some text-book writers have done. Fig. 4 shows this.

⁴ By the fissure of v. Ebner each sclerotome is divided into two portions, of which the posterior in the higher vertebrates plays the chief rôle in vertebral differentiation. "Scleromere" therefore seems an appropriate designation for the condensed sclerogenous tissue of this half-segment. Goette has recently, 97, brought forward evidence in favor of the view that primarily in the digitates there were two vertebrae to each body-segment. In the higher vertebrates, during embryonic development, the posterior skeletal area of each body-segment alone develops freely. The anterior area becomes fused with the scleromere in front.

This figure represents a horizontal section passing through several spinal ganglia, myotomes and neural processes. The last may be seen extending gradually into the area opposite the myotomic septa, but they still cover the whole posterior half of the median surface of the myotome in the region of the section. The processes are connected by membranous thickenings of the mesenchyme of the anterior half of each segment. These membranes may be called *interdorsal membranes*. They correspond to the *interdorsalia* of elasmobranchs. Figs. 12, 13, 15 and 16, *Idr. M.*, represent these membranes. A line drawn from A to B in Fig. 12 would pass through an area corresponding to that of the section represented in Fig. 4.

In the region where the neural and costal processes spring from the primitive disks membranous septa are likewise differentiated from the anterior halves of the sclerotomes. These septa serve to unite the successive disks. Each is continuous posteriorly with a dense tissue which strengthens the primitive disk and anteriorly it extends into the neural and costal processes. The relations of these interdiscal membranes are shown in Figs. 3, 11, 12 and 13, *Ids. m.* Since at this period structural outlines are by no means sharp, the figures based upon wax-plate reconstructions must be taken as semi-diagrammatic. A line drawn from *c* to *d* in Fig. 12 would represent essentially the plane of the section shown in Fig. 3.

During the development of the interdiscal membranes the primitive disks become hollowed out on the posterior surface. A comparison of Fig. 2 with Fig. 3 demonstrates this. The perichordal sheath meanwhile is developed in a ventrodorsal direction so that the area between the primitive disks becomes divided into right and left halves. Figs. 11, 13 and 7 all show this. Each lateral area is filled with a lightly staining mesenchyme which is continuous ventrally and dorsally with the tissue surrounding the spinal column.

Fig. 17, Plate V, represents a sagittal section cut slightly obliquely through an embryo 12 mm. long. In the region where the chorda (*Ch. d.*) is cut, the primitive disks may be seen united by a fairly dense tissue, the perichordal septum (*Sptm.*). Posterior to this region the section passes to one side of the chief axis of the embryo. The intervertebral disks may here be seen separated by a lighter tissue and in the more posterior portion of the section, which passes still more lateral to the chordal region, the tissue between the disks is seen to be continuous with that surrounding the spinal column. In this region the interdiscal membrane (*Ids. m.*) is seen anterior, the primitive disk posterior to the fissure of v. Ebner (*F. v. E.*).

Meanwhile the ventral processes of the thoracic vertebrae extend well into the thoracic wall, giving rise to primitive ribs, illustrated in Fig. B, Plate II, in the article of Bardeen and Lewis, or.

Development proceeds rapidly. In Embryo CIX, length 11 mm., age about five weeks (Figs. 14-16), the conditions are as follows: The neural processes are somewhat better developed than those of the preceding stage, but otherwise are similar in character. The costal processes are considerably farther developed (Bardeen and Lewis, or, Plate V, Fig. E). At the angle between the neural and costal processes opposite where they join the primitive disks a transverse process, but slightly indicated at the preceding stage, is now fairly clearly marked. Each primitive disk has become further hollowed out at its posterior surface, owing, in all probability, to the conversion of its tissue into that of the area between the disks. The interdiscal membrane (*Ids. m.*), on the other hand, has become thicker and has extended anteriorly and posteriorly about the area between the disks so that this has become completely enclosed (Figs. 14, 15 and 16). The tissue of each segment immediately anterior to the primitive disk has become greatly thickened and the line between it and the disk indistinct.

The area between each two primitive disks is still divided by the perichordal septum (Fig. 7). Each half represents the anlage of a chondrogenous center of the vertebral body. Formation of cartilage has not, however, begun. The thickening of the ventral margin of the primitive disk at this stage represents the "hypochordal Spange," which Froriep has shown to play an important part in the development of the vertebrae of birds and of the atlas in mammals. It has merely a transitory existence in the thoracic region of man.

Summary.—To sum up briefly, we may say that during the blastemal period each scleromere becomes divided into two portions, an anterior and a posterior, characterized by a much greater condensation of tissue in the posterior. From this condensed tissue arises a primitive vertebra of Remak, or scleromere, with dorsal (neural) and ventral (costal) processes and a disk uniting them to the mesenchyme condensed about the chorda dorsalis. From the tissue of the anterior half of each sclerotome arise membranes which serve to unite the dorsal processes of the scleromeres, *interdorsal membranes*, and to cover in the areas between the successive disks, *interdiscal membranes*. The primitive disks become hollowed out posteriorly by a loosening up of their tissue and strengthened anteriorly by a condensation of the tissue immediately bounding the fissure of v. Ebner. The area between each two disks is bilaterally divided by a membrane springing from the perichordal sheath. The formation of a cartilagenous skeleton now begins.

CHONDROGENOUS PERIOD.

The tissue relations during this period have been carefully studied in representatives of most of the chief groups of vertebrates. The form of the early structures has been less accurately determined because most investigators have avoided the somewhat laborious methods of plastic reconstruction.

On each side of the blastemal vertebra three primary centers of chondrification appear at about the same time, one for neural process, one for the costal process and one for the vertebral body. Fig. 7, Plate II, shows these centers as they appear in a cross section at an early period. Figs. 25, 26 and 27, Plate VI, show the early cartilages of an embryo slightly older, CXLIV, length 14 mm., age 5½ weeks.

The cartilages of the vertebral body develop by a transformation of the tissue lying between the primitive vertebral disks and surrounded by the interdiscal membrane. A considerable part of this tissue is derived from the posterior surface of each primitive disk. At first the cartilage of the left side is separated from that of the right by the perichordal septum. Soon this is broken through and the two anlagen of cartilage become united about the chorda. In the thoracic region this union seems to take place at about the same time dorsally than it does ventrally. A sagittal section of an embryo at this stage is shown in Fig. 18. The chorda dorsalis is surrounded by a perichordal sheath. The latter is encircled by dense intervertebral disks which alternate with light cartilagenous rings. The latter are surrounded by perichondrium which is less condensed than the tissue of the disks, but more so than that of the bodies and about the same as that of the perichordal sheath. Ventrally and dorsally a longitudinal ligament has been differentiated from the surrounding mesenchyme.

It is probable that the disks seen in this section are formed in part from the primitive disks, in part from the posterior layer of the anterior sclerotome halves; in other words, that each is formed about the rudiment of the fissure of v. Ebner. Compare Figs. 17 and 18. The tissue is concentrically arranged in a way somewhat resembling that of the intervertebral disks of the adult.

The perichordal tissue rapidly decreases in thickness. At the same time the cartilage of the vertebral bodies grows also at the expense of the intervertebral disks (Figs. 19, 20, 21 and 22). According to Schultze, 96, the cartilages of the bodies finally fuse to form a continuous cartilagenous column. This does not seem to be the case in man. In all of the embryos belonging to the collection of Prof. Mall some membranous tissue may be seen separating completely the successive bodies,

but in embryos between 20 and 40 mm. in length this membrane in the vicinity of the chorda dorsalis is very thin. At the periphery of the disks the annulus fibrosus is meanwhile differentiated more and more into a condition resembling the adult (Figs. 17-23, Plate V).

The chorda dorsalis at the period shown in Fig. 18 is of about the same size at the level of the disks and between them, but as the bodies increase in size at the expense of the disks the chordal canal becomes enlarged in the intervertebral areas and constricted at the centers of the bodies (Figs. 19, 20 and 21). The chorda loses its continuity and the chordal cells become clumped in the vicinity of the disks (Figs. 21 and 22) and finally spread out there in the form of a flat disk (Fig. 23). At this last period the perichondrium of the bodies is again becoming well marked and the portion of each intervertebral disk in the vicinity of the chorda dorsalis is better developed than during the stages immediately preceding. The chordal canal long remains in the vertebral body (Figs. 23 and 24).

The cartilage of the bodies in Embryo CXLIV (Fig. 18) is of an early embryonic hyaline type. At a slightly later stage (Fig. 19) two regions may be distinguished, a central and a peripheral. The central cartilage is denser than that of the preceding stage, while the peripheral cartilage resembles it. Gradually the cartilage at the center of the body undergoes further changes. The cells enlarge and become sharply set off against the intercellular substance (Figs. 22, 23 and 24), and finally an invasion of blood vessels takes place, chiefly from the posterior surface (Fig. 23). These changes in the cartilage, represented also in Fig. 41, Plate VII, are preliminary to ossification.

Deposit of calcium salts and actual ossification begins in the distal thoracic and proximal lumbar vertebrae of embryos about 5 to 7 cm. long and three months of age. Fig. 42 shows a center of ossification in an embryo of 70 mm.

During the development of the vertebral bodies changes have been active in the neural cartilages. At the period represented in Fig. 7, Plate II, the neural cartilage is a small, flat body situated in the dorsal process of the scleromere; from this as a center, pedicular, transverse, anterior (superior) and posterior (inferior) articular, and laminar processes are rapidly developed. This structural differentiation is best followed in the figures representing the models (Figs. 25-36). The *pedicular processes* are at first slender rods (Fig. 26), each of which grows out towards and finally fuses with its corresponding vertebral body. Froriep has shown (83) that in the chick this process forms a more essential element of the body than in mammals. In the atlas it forms a lateral half of the

ventral arch, but in the thoracic region of mammals it fuses with the antero-lateral portion of the corresponding vertebral body. After its junction with this the pedicle increases in size but otherwise shows no marked alteration of form.

The *transverse process* is at first a short projection which lies at some distance from its corresponding rib (Fig. 26). The cartilagenous rib rapidly increases in size and at the same time the transverse process grows outward and forward to meet it (Figs. 29, 32 and 34). At first the developing cartilage of the rib and that of the transverse process are embedded in a continuous blastema, but before chondrification has proceeded far, branches from successive intervertebral arteries become anastomosed in the area between the neck of the rib and the transverse process and separation is effected (Figs. 36, 38 B and 39).

Between the extremity of the transverse process and the rib a joint is developed (Figs. 39, 40, 41 and 42), and the surrounding blastema converted into costo-transverse ligaments.

The articular processes develop slowly from the cartilage. Extension takes place anteriorly, *A. A. Pr.*, and posteriorly, *P. A. Pr.*, in the interdorsal membrane. In an embryo of 14 mm. (Figs. 25, 26 and 27) these articular plates are separated by a distinct interval. In one of 17 mm. they have approached each other very closely (Fig. 37); and in one of 20 mm. not only do the articular processes show distinctly more form (Figs. 28, 29 and 30), but in addition the superior articular process slightly overlaps the inferior (Fig. 38). This overlap of the superior articular processes is distinctly more advanced in an embryo of 28 mm. (Fig. 39), and still more so in one of 33 mm. (Figs. 31-33). In an embryo of 50 mm. (Figs. 34, 35 and 40) conditions essentially like the adult have been reached.

The laminar processes scarcely exist in Embryo CXLIV (Fig. 26). In Embryo XXII (Fig. 29) they have begun to project posteriorly to the region of the articular processes (Fig. 29). The dense embryonic connective tissue covering the laminar processes at this stage gives attachment to a membrane covering the dorsal musculature, *F. D. M.*, and to a membrane surrounding the spinal cord, *M. R. D.* This accounts for the two projections seen dorsally on the side of the model representing the membranous tissue. In Embryo CXLV, length 33 mm., the laminar processes extend well toward the dorsal line (Figs. 32 and 33); in Embryo LXXXIV, length 50 mm. (Figs. 34, 35 and 40), they completely encircle the spinal canal and from the region of fusion of each pair a spinous process extends distally, though not so far as in the adult.

Alterations in the cartilage of the neural processes preliminary to

ossification begin at about the time they take place in the vertebral bodies. They are first seen in an area which corresponds to that in which the neural cartilage begins. The earliest calcification appears in Embryo CLXXXIV, length 50 mm., in the arches of the first cervical to the sixth thoracic vertebrae.

The development of the ribs I shall not attempt in this place to describe in detail. Figs. 25-34 and 37-42 show sufficiently well the relations of the proximal ends of the ribs to the vertebrae. They are developed opposite the intervertebral disks. The blastemal tissue which surrounds the developing heads of the ribs becomes converted into costo-vertebral ligaments. Differentiation in the cartilage preliminary to ossification takes place in the shafts of the ribs even earlier than in the vertebral bodies and in the neural processes. Ossification is well under way in the shafts of the ribs of Embryo LXXIX, length 33 mm.; XCVI, length 44 mm.; XCV, length, 46 mm.; and LXXXIV, length 50 mm.

Summary of the Chondrogenous Period of Vertebral Development.—Each cartilagenous vertebra is developed from four centers of chondrification. In addition, a separate center appears for each rib. In comparing these centers with the blastemal formative centers, we find that each primitive center of blastemal condensation enters into union with tissue derived from the anterior half of the body-segment next posterior and then gives rise to three centers of chondrification, one for the neural arch, one for the rib and one for half a vertebra. When ossification first takes place the centers for the ossification of the neural arches and the ribs correspond to the original chondrification centers in the blastema, but the centers for ossification of the bodies show little trace of the bilateral condition which marks the cartilagenous fundaments.

The processes of chondrogenous form differentiation are shown in the drawings of the models. The period of ossification of the vertebrae has been so often and so well described that no attempt will be made to enter upon a further account of it in this paper. I have, however, not found two primary ossification centers, such as Renault and Rambaud have described, for each neural arch.

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ABBREVIATIONS USED TO DESIGNATE STRUCTURES ILLUSTRATED
IN THE FIGURES.

<i>A. A. Pr.</i> , anterior articular process.	<i>N. Pr.</i> , neural process.
<i>C. V.</i> , cardinal vein.	<i>Pch. S.</i> , perichordal sheath.
<i>C. Pr.</i> , costal process.	<i>P. A. Pr.</i> , posterior articular process.
<i>Cœl.</i> , cœlom.	<i>Pd.</i> , pedicle.
<i>Ch. d.</i> , chora dorsalis.	<i>Rib.</i> , rib.
<i>Derm.</i> , dermis.	<i>Scl.</i> , sclerotome.
<i>Disk.</i> , intervertebral disk.	<i>Sptm.</i> , perichordal septum.
<i>D. L.</i> , dorsal ligament.	<i>Sp. C.</i> , spinal cord.
<i>D. M.</i> , dorsal musculature.	<i>Sp. G.</i> , spinal ganglion.
<i>F. v. E.</i> , fissure of v. Ebner.	<i>Sp. N.</i> , spinal nerve.
<i>F. D. M.</i> , fascia of dorsal musculature.	<i>Sp. Pr.</i> , spinous process.
<i>Ids. M.</i> , interdiscal membrane.	<i>Trap.</i> , trapezius.
<i>Idr. M.</i> , interdorsal membrane.	<i>Tr. Pr.</i> , transverse process.
<i>Is. A.</i> , intersegmental artery.	<i>V. L.</i> , ventral ligament.
<i>L.</i> , lamina.	<i>V. B.</i> , vertebral body.
<i>Myo.</i> , myotome.	5, 6, 7, 5th, 6th and 7th thoracic vertebræ.
<i>M. R. D.</i> , membrana reuniens dorsalis.	

EXPLANATION OF FIGURES.

PLATE I.

Figs. 1, 2, 3 and 4. Frontal sections through the thoracic region of several embryos during the blastemal period of vertebral development. 47.5 diam. (1) Embryo CLXXXVI, length 3.5 mm. (2) Embryo LXXX, length 5 mm. (3) and (4) Embryo CCXLI, length 6 mm. (3) Through the region of the chorda dorsalis, (4) through a more dorsal plane. Figures 1, 3 and 4 represent sections cut somewhat obliquely so that the right side of the sections is ventral to the left. In Figs. 2 and 4 on the right side the bodies of several embryonic vertebræ are represented in outline. In Figs. 2 and 3 owing to artefacts the myotomes are pulled away from the sclerotomes.

PLATE II.

Figs. 5, 6 and 7. Cross-sections through midthoracic segments during the blastemal period of vertebral development. 55 diam. (5) Embryo LXXVI, length 4.5 mm. The right side of the section passes through the middle, the left side through the posterior third of the 5th segment. (6) Embryo II, length 7 mm. 5th thoracic segment. The right side of the drawing represents a section anterior to that shown at the left. (7) Embryo CLXXV, length 13 mm. The left half of the 6th vertebral body, neural process and rib are drawn in detail, the body-wall, spinal cord and spinal ganglion are shown in outline.

PLATES III AND IV.

Figs. 8, 9, 10, 11, 12 and 13. Views of models representing the blastemal stage of vertebral development. (8-10) Embryo II, length 7 mm., 33½ diam. (11-13) Embryo CLXIII, length 9 mm., 25 diam. (14-16) Embryo CIX, length 11 mm., 25 diam. 8, 11 and 14 views from in front; 9, 12, 15, views from the side; 10, 13, 16, views from behind.

PLATE V.

Figs. 17-24. Sagittal sections in the mid-line through the 6th, 7th and 8th thoracic segments of a series of embryos from 12 to 50 mm. long. (17) Embryo CCXXI, length 12 mm. This section includes several segments anterior and posterior to the three above mentioned, 6th, 7th and 8th. (18) Embryo CXLIV, length 14 mm. (19) Embryo CVIII, length 22 mm. (20) Embryo LXXXVI, length 30 mm. (21) Embryo CXLV, length 33 mm. (22) Embryo LXXIX, length 33 mm. (23) Embryo XCVI, length 44 mm. (24) Embryo CLXXXIV, length 50 mm.

PLATE VI.

Figs. 25-35. Dorsal, lateral and ventral views of models made by the Born method to illustrate vertebral form-differentiation in the 6th, 7th and 8th thoracic vertebrae during the chondrogenous period. On the left side the cartilagenous, on the right the enveloping fibrous tissue is shown. The latter is also shown on the eighth vertebra in Figures 29 and 35. (25-27) Embryo CXLIV, length 14 mm., 20 diam. (28-30) Embryo XXII, length 20 mm., 13 diam. (31-33) Embryo CXLV, length 33 mm., 10 diam. (34, 35) Embryo LXXXIV, length 50 mm., 10 diam. (34) Dorsal view, left half; (35) median view.

PLATE VII.

Figs. 36-42. Transverse sections through mid-thoracic vertebrae of a series of embryos. 5 diam. (36) Embryo CVI, length 17 mm. (37) Embryo CCXVI, length 17 mm. (38) Embryo XXII, length 20 mm. (39) Embryo XLV, length 20 mm. (40) Embryo LXXXIV, length 50 mm. (41) Embryo XLIV, length 70 mm. (42) Embryo XXIII, length 70 mm.

The models from which the illustrations in this article were drawn have been reproduced by Dr. B. E. Dahlgren at the American Museum of Natural History, New York, N. Y., and arrangements may be made for securing copies by applying to the Director of the Museum.

THE DEVELOPMENT OF THE THORACIC VERTEBRAE IN MAN

C. R. BARDEEN

PLATE I

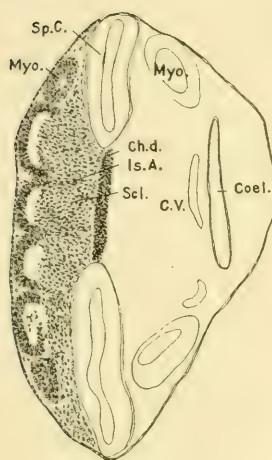


Fig. 1

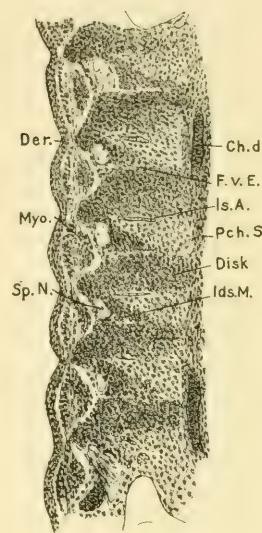


Fig. 3

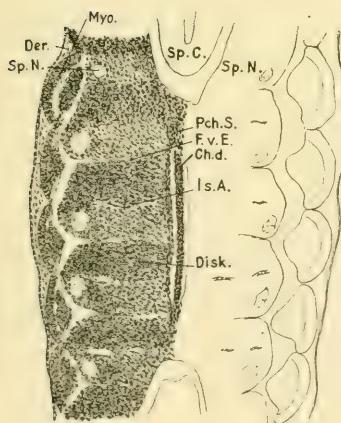


Fig. 2

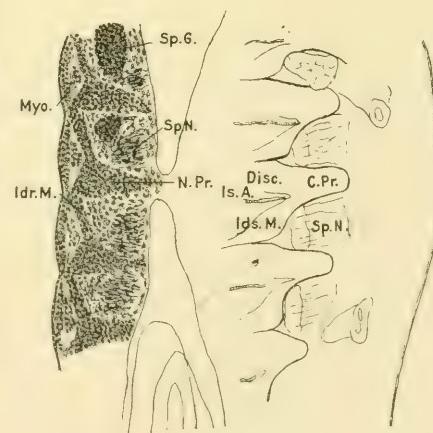
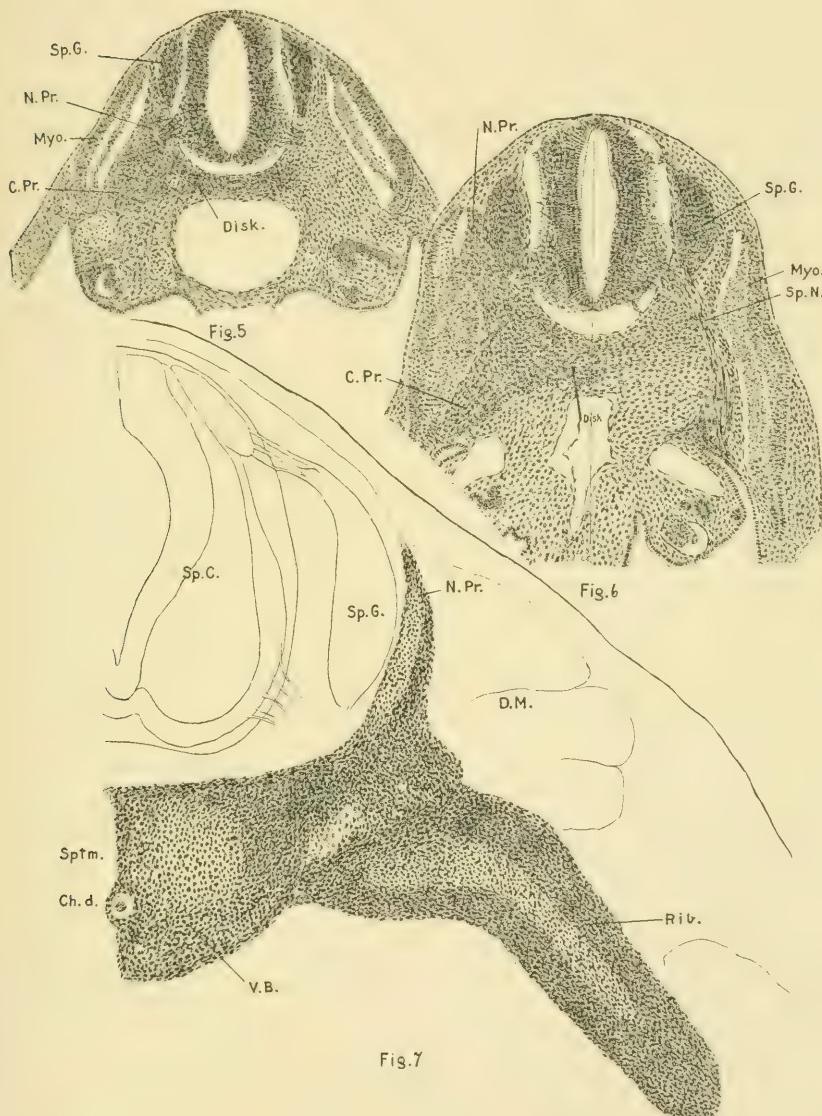


Fig. 4

THE DEVELOPMENT OF THE THORACIC VERTEBRÆ IN MAN

PLATE II

C. R. BARDEEN



THE DEVELOPMENT OF THE THORACIC VERTEBRAE IN MAN

C. R. BARDEEN

PLATE III

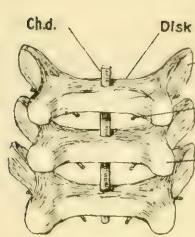


Fig. 8

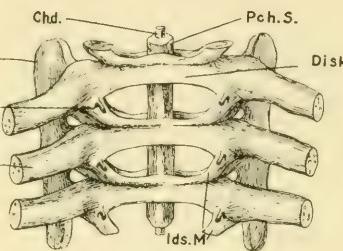


Fig. 11

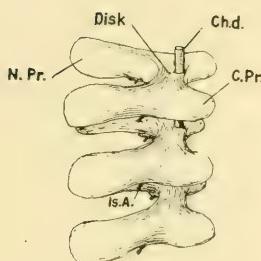


Fig. 9

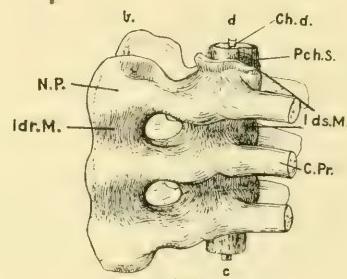


Fig. 12.

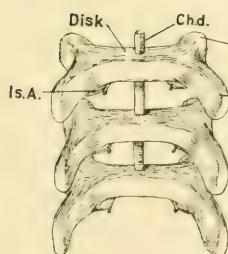


Fig. 10

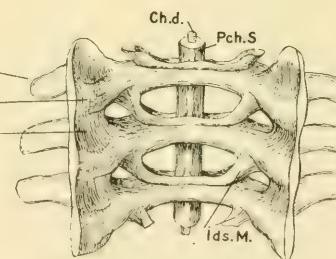


Fig. 13

THE DEVELOPMENT OF THE THORACIC VERTEBRÆ IN MAN

PLATE IV

C. R. BARDEEN

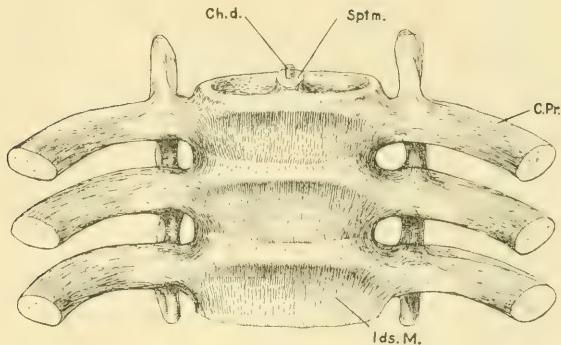


Fig. 14

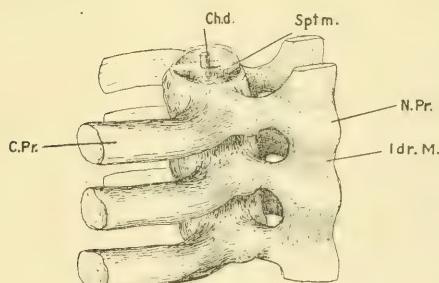


Fig. 15

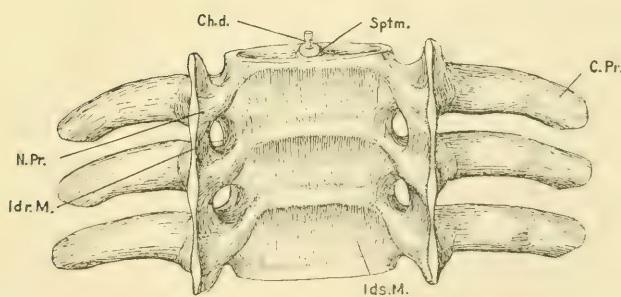


Fig. 16

THE DEVELOPMENT OF THE THORACIC VERTEBRAE IN MAN

C. R. BARDEEN

PLATE V

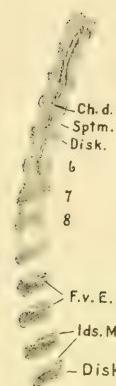


Fig.17



Fig.18



Fig.20.



Fig.21

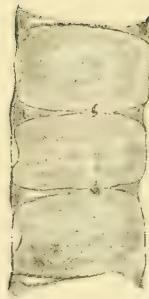


Fig.22

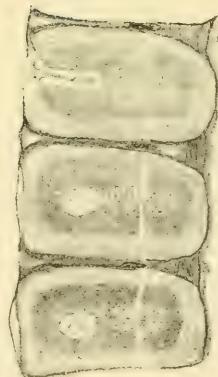


Fig.23

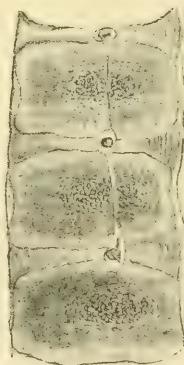
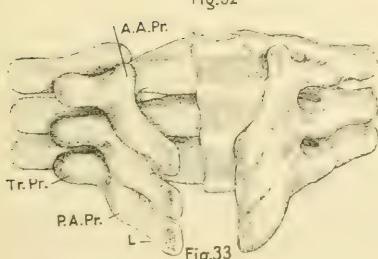
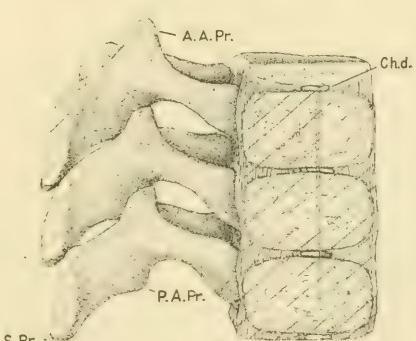
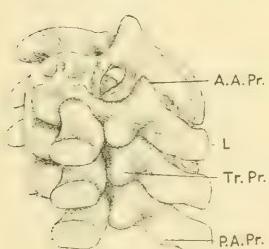
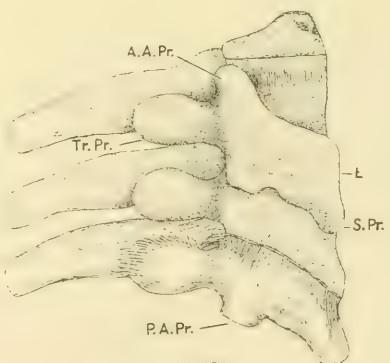
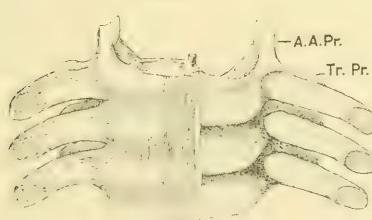
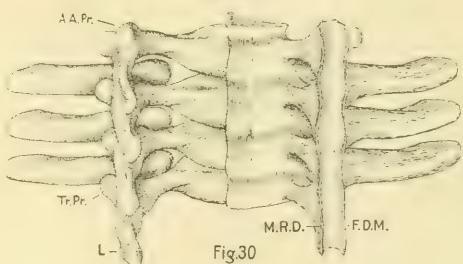
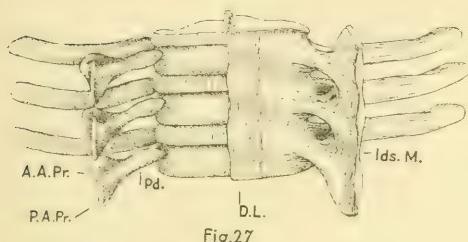
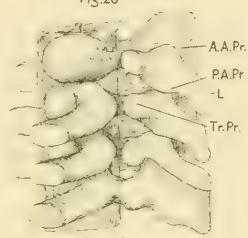
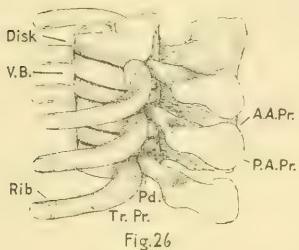
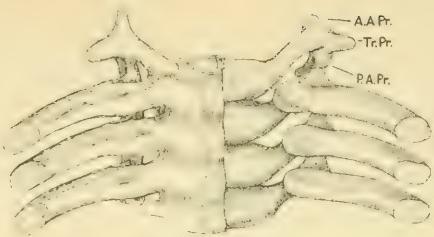
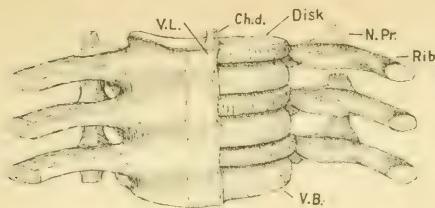


Fig.24

C. R. BARDEEN



THE DEVELOPMENT OF THE THORACIC VERTEBRAE IN MAN

PLATE VII

C. R. BARDEEN

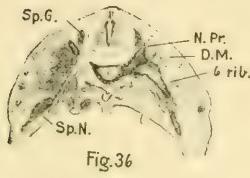


Fig. 36

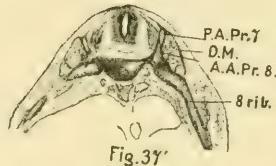


Fig. 37

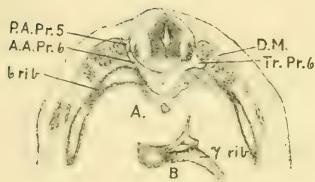


Fig. 38

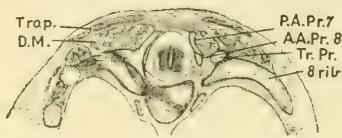


Fig. 39



Fig. 40

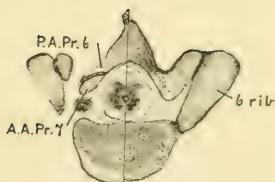


Fig. 41

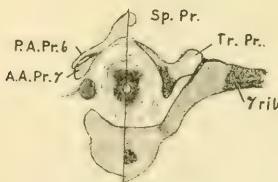


Fig. 42

THE ELASTIC TISSUE OF THE HUMAN LARYNX.

BY

DEAN D. LEWIS, M. D.

From the Hull Laboratory of Anatomy, University of Chicago.

WITH 5 PLATES.

The elastic tissue of the larynx, since it was first described by Lauth,¹ in 1835, has been the object of frequent studies by anatomists and laryngologists. The functional importance of this tissue in the production and modification of voice has aroused interest in its distribution and arrangement, and while the earlier descriptions, which are based upon the study of specimens prepared by methods which do not reveal the finer elastic fibrils, are correct, a knowledge of the more minute relations existing between the elastic fibers, muscle, cartilage and epithelium is desirable.

The introduction of specific stains for elastic tissue has given a new impetus to its study from a developmental and pathological view-point, and has drawn attention to the elastic tissue of the larynx, as is evidenced by the number of articles appearing upon the subject. Friedrich's work followed closely upon the introduction of the Taenzer-Unna orcein stain, and Katzenstein, using the Weigert resorcin-fuchsin method, has repeated recently the former's work. The Weigert method certainly differentiates the elastic fibers more distinctly than the Taenzer-Unna method, and stains the finer fibrils, which may escape the latter. These two investigators agree in general, but differ in so many points that the publication of this article, which was about completed when Katzenstein's article appeared, seems justified.

The specimens from which this study is made were prepared from the larynges of the new-born. They were hardened in alcohol and Zenker's fluid. The former fixative, recommended by Weigert, gives excellent results, but equally good results are obtained after fixation in Zenker's fluid. The ordinary celloidin technique was used, with the addition of

¹ Lauth: Mem. de l'acad. royale de med., 1835, t. 4, p. 98.

the slow method of celloidin infiltration, as recently recommended by Miller.²

Sections, fifteen micra in thickness, were made and mounted serially. The sections were passed through a mixture of alcohol and glycerine, from which they were transferred to a paper corresponding in size to the cover-glass to be used. The paper was then inverted upon a slide, which had previously been coated with a thin layer of albumin fixative. Between two slides thus prepared a piece of filter paper was inserted, and the two slides were tied together. They were then placed in a thermostat until dry, when they were removed and placed in equal parts of absolute alcohol and ether to remove the celloidin. The sections are always firmly attached, and no care need be exercised to prevent their floating off. This method was devised by Prof. E. C. Jeffrey. Weigert's resorcin-fuchsin method was used to stain the elastic fibers. Orange G. as a counter-stain offers a sharp contrast to the blue-black of these fibers. Van Gieson's picro-fuchsin was used in studying the collagenic fibers and the nodules found in the anterior extremities of the vocal cords.

Henle³ describes beneath the mucous membrane of the larynx an elastic fiber layer, which in some regions is poorly, and in others well developed, and in some closely and in others loosely connected with the epithelium. Where this layer is thickened after removal of the mucous membrane or the tissue which covers them externally, there remain ligaments. These ligaments are attached at definite points to the perichondrium of the laryngeal cartilages, and such points of attachment may be regarded as the points of origin of the ligaments. It is not to be disregarded, however, that the elastic fibers of these ligaments are in direct continuity with the elastic elements of the whole mucous tract, and that, therefore, their limits are not sharp and are arbitrarily made.

Following the classical description of Luschka⁴ the elastic tissue of the larynx may be divided for descriptive purposes into three zones, corresponding to the three compartments of this organ. The inferior zone includes all the elastic tissue within and below the *ligamenta vocalia*; the middle zone includes the elastic tissue surrounding the *ventriculus laryngis*; the superior zone includes the elastic tissue of the *membrana quadrangularis* and *epiglottis*. The discussion of the arrangement of the elastic fibers will be proceeded with in the order given above.

² Miller: J. of Applied Microscopy and Laboratory Methods. Rochester, N. Y., Vol. 6, No. 4.

³ Henle: Handb. der Eingeweidelehre des Menschen. Braunschweig, 1873, p. 254.

⁴ Luschka: Der Kehlkopf des Menschen. Tübingen, 1874.

ELASTIC TISSUE OF INFERIOR ZONE.

CONUS ELASTICUS (LUSCHKA) : LIGG. CRICO-THYREO-ARYTENOIDEA
OF KRAUSE⁵ AND LIG. CRICOHYREOIDEUM MEDIUM (HENLE).

If a lamina of the thyroid cartilage and the subjacent muscle be removed, a fan-shaped mass of elastic fibers will be seen, which passes from the angle of the thyroid cartilage downward, backward and laterad to be attached to the ascending upper border of the cricoid cartilage and the inferior surface of the vocal process of the arytenoid cartilage. This elastic membrane is the conus elasticus of Luschka. Sections at various levels through it will be described.

In frontal sections, made through the anterior part of the conus elasticus, a dense network of elastic fibers arising from the upper border of the cricoid cartilage, and passing cephalad and laterad to be attached to the lower border of the thyroid cartilage, will be seen. The fibers composing this network tend to pass vertically, anastomose freely, and some of the fibers arise on each side of an indefinite median raphé. On both sides these fibers are continuous with elastic fibers passing from the upper border of the cricoid cartilage. The mass of elastic fibers occupying the median line form the ligamentum cricothyreoideum medium, and are seen to be merely the anterior continuation of the conus elasticus, as previously shown by His. This ligament is pierced by the cricothyroid artery, and the arrangement of some of its component fibers about a median raphé suggests that functionally it is divided into symmetrical parts. (See Fig. 1.)

In frontal sections made through the middle of the conus elasticus, elastic fibers, few in number, and small in size, are seen arising from the upper border of the cricoid cartilage, and passing cephalad and mediad to reach the ligamentum vocale. These fibers, increasing constantly in number and size as they ascend, form a gentle curve, the convexity of which is directed mediad, being separated in the subglottic region from the subepithelial elastic layer, by numerous glands, and loose connective tissue, which favors the development of edema at this point. The concavity is occupied by the *musculus vocalis*. The fibers below are obliquely arranged, and only as the plica vocalis is approached do they tend to become sagittally directed and parallel. (See Fig. 2.)

Sections made through the posterior part show that the conus becomes shorter and approaches nearer the median line. The fibers are more nearly vertical in arrangement, passing up to the inferior surface of the

⁵ Krause: Handb. der menschlichen Anatomie. Hannover, 1879.

vocal process of the arytenoid cartilage. While these sections are instructive, much more may be learned by tracing transverse sections serially.

In transverse sections made through the ligamentum cricothyreoideum medium, the elastic fibers are found grouped in well-defined vertical bundles, which are separated from each other by horizontal fibers passing inward toward the indefinite median raphé. Fine elastic fibers, vertically directed, which surround numerous groups of mucous glands, pass from the subepithelial layer to the ligament. (See Fig. 4.)

More posteriorly the fibers of the conus pass obliquely, upward, forward and mediad, but they are intersected by other fibers passing almost at right angles to them. The oblique fibers predominate, both in size and number. Traced backward, the elastic fibers of the conus are found to be attached to the cricoid cartilage. Anastomosing fibers, most numerous anteriorly, connect the fibers of the conus with the subepithelial layer, where the two systems are not separated by the glands previously mentioned. (See Fig. 5.)

In transverse sections just below the ligamentum vocale, the arrangement of elastic fibers anteriorly has been accurately described by Friedrich. The elastic tissue, reduced in amount, is replaced by collagenic fibers, which separate the fibers of the conus from the hyaline substance of the thyroid cartilage. This collagenic tissue is rich in glands. The nearer the ventricle is approached, the more nearly horizontal the fibers become to form the

LIGAMENTUM VOCALE. (*See Fig. 7.*)

Henle,⁶ in describing the ligamentum vocale, states that some of its elastic fibers fuse posteriorly with the elastic cartilage, forming the vocal process of the arytenoid. Other fibers are attached about the spina inferior, above the vocal process, and from this attachment fibers course upward posterior to the ventricle of the larynx. Still other fibers are inserted below the vocal process upon the medial surface of the arytenoid cartilage, or upon the anterior surface of the cricoid cartilage.

Kanthack⁷ describes posteriorly a sesamoid cartilage, which marks the point of transition of the elastic fibers of the ligamentum vocale into the vocal process of the arytenoid cartilage.

Reinke's⁸ description of the arrangement of the elastic fibers at the posterior extremity of the ligamentum vocale, and their relation to the

⁶ Henle: *Handb. der Eingeweidelehre des Menschen*; p. 255.

⁷ Kanthack: *Arch. f. path. Anat.*, etc., Berl., Bd. cxvii, p. 533.

⁸ Reinke: *Anat. Hefte*, Bd. ix, pp. 108-110.

processus vocalis of the arytenoid cartilage, is the most accurate that has yet been given. His findings can merely be verified; nothing can be added. He states that in macroscopical preparations it can be seen that the ligamentum vocale has a wide area of attachment to the vocal process of the cartilage, covering its upper and medial surfaces, leaving the lower and lateral surfaces free for the attachment of the fibers of the musculus vocalis. The study of microscopical preparations made in frontal and horizontal planes shows that the middle fibers of the ligament, only, are the direct continuation of the fibers of the elastic cartilage, which forms the apex and anterior border of the vocal process. The greater number of fibers, occupying the lateral part of the ligament, are derived from the perichondrium, which consists here almost wholly of elastic fibers, which cover the elastic cartilage above, laterally and medially.

In the beginning of the elastic cartilage the fibers intersect each other at various angles, but soon pass parallel in a sagittal plane. The fibers of the perichondrium upon the medial and lateral surface of the cartilage pass in front of the apex and anterior border of the processus vocalis in curves, which intersect each other at right angles. All these fibers form anterior to the vocal process a dense network, probably the sesamoid cartilage of Kanthack, out of which parallel fibers emerge, to pass forward. The fibers of the ligament receive from the side additional fibers, which lie between the bundles of the musculus vocalis, and by means of its perimysium they are attached to the vocal process of the arytenoid cartilage.

In the middle part of the ligament the fibers usually course parallel to each other, and in a sagittal direction. When the processus vocalis is in the position of rest, two great divisions of the ligament may be differentiated. The one adjacent to the muscle is dense and only in thin sections can the separate fibers be differentiated; the other, equally wide, borders upon the preceding, above and medially, and has the same form. In sections, it is recognizable as a lighter zone. The fibers of the denser part of the ligament form a curve, the concavity of which is directed toward the free border of the labium vocale, while the fibers of the less compact part are straight.

Upon superficial examination, the fibers of the ligament seem to pass parallel to each other, without anastomosing. C. L. Merkel has stated that the elastic fibers of the ligamentum vocale differ from the elastic fibers in other parts of the body, in that they do not anastomose. Reinke has shown, however, by the use of specific stains and higher lenses that there are anastomosing fibers, but that the principal fibers are so well

developed that it is difficult to see the finer anastomosing ones. Reinke's description is most accurate, and he has demonstrated that the elastic fibers of the ligament have a definite structure, relative to their function. His conclusions may be summed up as follows: The elastic fibers of the ligamentum vocale attain their greatest development in planes parallel to constant tension, and at right angles to constant pressure, while the fibers passing obliquely to anastomose with either of the above systems remain atrophic or have disappeared.

THE ANTERIOR ATTACHMENTS OF THE LIGAMENTA VOCALIA.

There is so much difference of opinion among anatomists and laryngologists concerning the anterior attachment of the ligamentum vocale, that some account of the views held by the different investigators may be expedient.

C. Mayer⁹ was the first to describe in the anterior extremity of each ligament a small cartilaginous nodule, which was found by him in man, and some of the higher apes. These nodules are sometimes designated as the "cartilagine sesamoideæ anteriores." (See Fig. 8.)

Gerhardt¹⁰ noted in sections made at the level of the insertion of the ligamenta vocalia into the thyroid cartilage a small firm median process, occupying the angle of the thyroid cartilage, which he considered to be formed from its hyaline substance. This median process is prolonged on each side by yellowish flexible bands into the anterior extremities of the ligamenta vocalia. The yellowish color of the anterior commissure and its thickening is produced by these processes. The intimacy of the relation between the median process and its lateral prolongations is variable. However, Gerhardt macerated sections many days in water, but was still unable to separate them without the use of a knife. He considered that microscopically there is repeated at the anterior commissure the same histological structure that Rheiner had previously described for the processus vocalis of the arytenoid cartilage, and suggested that this median process, with its lateral prolongations, be known as the processus vocalis of the thyroid cartilage.

Verson¹¹ notes that the ligamentum vocale is thickened to form a small nodule immediately behind its attachment to the angle of the thyroid cartilage. Upon sectioning this nodule, it is seen to be composed

⁹ Mayer, C.: J. F. Meckel's Arch. f. Anat. u. Physiol.; 1826, p. 194.

¹⁰ Gerhardt: Arch. f. path. Anat., etc., Berl., Bd. xix, pp. 436-437.

¹¹ Verson: Stricker's Handb. der Lehre von den Geweben des Menschen u. der Thiere. Leipzig, 1871, p. 460.

of elastic fibers, round and spindle cells. It is found in the new-born. Chondrification never occurs in it.

Sappey¹² states that the ligamentum vocale is attached anteriorly by means of a nodule composed of elastic tissue, "nodule glottique antérieur."

Fränkel¹³ describes in the anterior extremity of the ligamentum vocale a small firm nodule, which is attached to the hyaline thyroid cartilage by loose fibrous tissue. He states that the nodule undoubtedly contains cartilage cells.

Nicolas¹⁴ speaks of the "cartilagines sesamoideæ anteriores" as small white or yellowish-white nodules, which can be isolated without difficulty from the fibro-elastic tissue in which they are lodged. They do not exceed a millet seed in size; often they are smaller. They are attached to the angle of the thyroid cartilage by a dense fibrous tissue. In the majority of cases, if not in all, among the elastic and collagenic fibers forming the nodules, cells are found which are undoubtedly cartilaginous in character.

Reinke¹⁵ describes the nodule as being composed of a deeply stainable substance, resembling histologically the tissue just anterior to the processus vocalis of the arytenoid cartilage. Some cartilage cells are found in the nodule. The elastic fibers passing off from the anterior extremity of this nodule are attached to the perichondrium of the thyroid cartilage.

None of the investigators had paid much attention to the finer histological structure of the median process described by Gerhardt, and it remained for Friedrich¹⁶ to study it in detail, and to question the conclusions of the former. Friedrich describes accurately the relation existing between the collagenic fibers, occupying the angle of the thyroid cartilage, and the anterior fibers of the conus elasticus. This fibrous tissue increases in amount as sections passing cephalad are examined; it attains its greatest development opposite the anterior attachments of the ligamenta vocalia. The fibrous tissue will be found grouped in well-defined vertical bundles immediately adjacent to the thyroid cartilage. These vertical bundles are surrounded by horizontal fibers. Posteriorly, as the anterior extremities of the ligamenta vocalia are approached, the bands of fibrous tissue become horizontal, corresponding in direction to a horizontal group of elastic fibers, passing off

¹² Sappey; quoted by Friedrich.

¹³ Fränkel: Arch. f. Laryngol. u. Rhinol. Bd. i.

¹⁴ Nicolas: Poirier et Charpy's Traité d'anatomie humaine., t. iv, p. 435.

¹⁵ Reinke: Anat. Hefte. Bd. ix, p. 110.

¹⁶ Friedrich: Arch. f. Laryngol. u. Rhinol. Bd. iv, pp. 192-193.

from the anterior extremities of the nodules described by Mayer. Interspersed throughout this fibrous tissue are some cartilage cells and fine elastic fibrils, which are vertically directed. (See Fig. 8.)

Friedrich states that the perichondrium passes between the hyaline substance of the thyroid cartilage and the median process described by Gerhardt, and that it forms a distinct line of separation between the two. He concludes, therefore, that there is no gradual transition from a hyaline to an elastic cartilage, as is the case in the arytenoid cartilage, and that Gerhardt is not justified in speaking of the process described by him, as the processus vocalis of the thyroid cartilage. Friedrich found no cartilage cells in the nodules occupying the anterior extremities of the ligamenta vocalia.

Katzenstein,¹⁷ while agreeing with Friedrich in many points, takes issue with him concerning the anatomical significance of the median process. He states that the perichondrium is reflected upon the sides of the process, and that it does not form a line of separation between the fibrous median process and the hyaline substance of the thyroid cartilage. In establishing the perichondrial relation, he makes use of the law first suggested by Rawitz, that the perichondrium exerts a directive influence upon the orientation of cartilage cells. Upon the external surface of the thyroid cartilage the cells are arranged parallel to the fibers of the perichondrium. In the center of the cartilage they lie at right angles to its long axis; upon the inner surface of the cartilage the cells are arranged parallel to the fibers of the perichondrium, until the median process is reached, where they are gathered into irregular clusters and some of the cartilage cells are displaced posteriorly. Katzenstein agrees with Gerhardt regarding the anatomical significance of this process, and considers it as quite comparable to the processus vocalis of the arytenoid cartilage. Like Reinke, in his work upon the ligamentum vocale, Katzenstein has shown that the fibers are arranged in definite tension planes.

In some of the lower animals, white rat, cat, etc., Katzenstein has described between the laminæ of the thyroid cartilage a wedge-shaped cartilage, which is covered by elastic fibers anteriorly, and receives posteriorly the attachment of the anterior extremities of the ligamenta vocalia. According to him, this wedge-shaped cartilage is the homologue of the median process described by Gerhardt.

While Katzenstein has accurately described the histological findings, he has misinterpreted their anatomical significance. If the developmental history of the thyroid cartilage be reviewed, some facts will be

¹⁷ Katzenstein: Arch. f. Laryngol. u. Rhinol. Bd. xiii, pp. 336-337.

met with which will explain the different orientation of cartilage cells adjacent to the process, and the significance of the wedge-shaped cartilage described by the investigator.

Rambaud and Renault,¹⁸ in discussing the development of the thyroid cartilage, say that the laminæ of the thyroid cartilage are united by means of a circumscribed median cartilage—"le cartilage vocal." It may be distinguished by its transparency. This cartilage is well-marked in young subjects. In the adult, however, the cartilage may not be present, but is represented by an indistinct point of ossification. It is lozenge-shaped, and its borders unite with the laminæ of the thyroid cartilage.

Henle¹⁹ states that horizontal sections through the thyroid cartilage show that its laminæ are separated more or less distinctly from a middle piece by a condensed layer of interstitial substance, curved so that the convexity is directed mediad. This middle piece of the thyroid cartilage is the lamina mediana cartilaginis thyreoidæ of Halbertsma. In this piece the cartilage nests are smaller and more closely grouped than in the laminæ. The ligamenta thyreo-arytaenoidea inferioria and their corresponding muscles arise from this part, or a connective tissue mass, connected with it. The fibers from the mass pass a short way into the middle piece, so that this tissue immediately posterior to the hyaline cartilage resembles in structure fibro-cartilage.

Nicolas²⁰ states that immediately after birth there is found in the median line of the thyroid cartilage, at the level of the vocal cords, a special arrangement of the cartilage cells, corresponding to the position of the lamina mediana. In the adult this middle portion of the thyroid cartilage can be distinguished from its laminæ only by the different orientation of its cells.

The embryology of the thyroid cartilage explains the different orientation of the cartilage cells adjacent to the median process, and in it. I have sectioned the larynges of young dogs, and have found, occupying the space between the laminæ of the thyroid cartilage, the wedge-shaped cartilage which Katzenstein states is comparable to the median process described by Gerhardt in man. I consider it to be the lamina mediana which fuses later in the lower animals than in man.

Friedrich's description of the median process is correct, with the exception of that of the perichondrial relation. The perichondrium does not pass between the hyaline substance of the thyroid cartilage and the

¹⁸ Rambaud et Renault: *Origin et developpement des os.* Paris, 1864.

¹⁹ Henle: *Handb. der Eingeweidelehre des Menschen;* p. 243.

²⁰ Nicolas: *Poirier et Charpy's Traite d'Anatomie humaine.* T. 4, p. 447.

fibrous tissue composing the process, nor is it reflected upon the sides of it, as described by Katzenstein. The whole process is formed by a thickening of the perichondrium, which passes directly backward to receive the attachment of the elastic fibers of the ligamenta vocalia. This is indicated by the arrangement of the elastic fibers of the perichondrium at this point, which, adjacent to the thyroid cartilage, are directed either antero-posteriorly or obliquely. The attachment of the elastic fibers of the ligamenta vocalia at this point is comparable to their attachment by means of the perichondrium to the laryngeal cartilage at other points. The great increase in the amount of elastic tissue in the ligamenta vocalia demands an increase in the number and size of the perichondrial fibers to which they are attached. This increase, gradual from below upwards, corresponds to the increase in the number of the fibers composing the conus elasticus, as it passes upward.

Adjacent to the thyroid cartilage, the fibers are grouped in vertical bundles, which are separated from each other by horizontal fibers. Posteriorly, the perichondrial fibers are collected into horizontal bundles, which are separated from each other by blood vessels, and ducts of glands, vertically directed. Into these parallel bands of fibrous tissue are attached parallel bundles of elastic fibers, which pass off from the nodules occupying the anterior extremities of the ligamenta vocalia. Scattered throughout the perichondrium at this point are fine elastic fibers, vertically directed. I have been unable to find cartilage cells as far posterior as described by Friedrich and Katzenstein. These cells seem to be restricted to a narrow zone immediately adjacent to the thyroid cartilage.

In specimens stained by Van Gieson's method, fibrous bands are seen to pass off posteriorly from perichondrium to surround a nodule, which occupies the anterior extremity of each ligamentum vocale, and which is composed of round and spindle cells. While histologically these nodules resemble somewhat those found anterior to the processus vocales of the arytenoid cartilages, I have been unable to find cartilage cells in them. (See Fig. 9.)

In specimens stained by Weigert's method, the elastic fibers of the ligamentum vocale will be seen to pass into the nodule, parallel to each other, posteriorly, while from its anterior extremity and medial surface several heavy bundles of anastomosing elastic fibers pass off, to be attached to the parallel fibers of the perichondrium. Some elastic fibers apparently originate in the nodule, for the fibers passing off from its anterior extremity exceed in number those passing into it posteriorly.

In conclusion, I agree with Friedrich in not considering the process

described by Gerhardt as the processus vocalis of the thyroid cartilage. It may be compared to the other perichondrial processes by which the elastic tissue of the larynx is attached to the laryngeal cartilages at different points. It is impossible to explain why the thyroid cartilage develops as it does, but the relation existing between the fibrous tissue occupying the concavity of the lamina mediana, and the elastic tissue of the ligamentum vocale does not resemble in the least the histological relations existing between the hyaline substance of the arytenoid cartilages, and their vocal processes.

I have not found cartilage cells in the nodules occupying the anterior extremities of the ligamenta vocalia. I would suggest that these nodules be known as the noduli vocales. It is difficult to assign to these nodules their physiological function, but the increase in the number of elastic fibers and their arrangement at this point, strengthened, as they are, by numerous round and spindle cells, would suggest that the ligamenta vocalia are here subjected to their greatest tension, and are therefore re-inforced.

THE RELATION OF THE MUSCULUS VOCALIS TO THE LIGAMENTUM VOCALE.

The relation of the musculus vocalis to the elastic fibers of the ligamentum vocale is highly important, and although it has been studied by many anatomists and laryngologists in recent years, there is at the present time no uniformity of opinion concerning it, or its functional significance in the production or modification of higher tones.

Ludwig²¹ describes the musculus thyreo-arytaenoideus as being divided into a portio aryvocalis and arythyreoidea. The former division begins upon the lower extremity of the anterior surface of the arytenoid cartilage, and passes in parallel bundles by the side of the ligamentum vocale, to end in it. The shorter fibers end directly anterior to the apex of the vocal process; the longer near the thyroid cartilage. These fibers, acting simultaneously, draw the ligamentum vocale downward and outward. If they act independently of each other, different segments of the ligament will be affected in different ways. Fibers anterior to the insertion of the muscle are rendered tense, while the fibers posterior to it are relaxed. Ludwig regards the ligamentum vocale as the tendon of the musculus thyreo-arytaenoideus.

Verson²² denies that any fibers of the musculus thyreo-arytaenoideus are inserted into the elastic fibers of the ligamentum vocale.

²¹ Ludwig: Lehrbuch der Physiologie der Menschen. Bd. i, pp. 567-570.

²² Verson: Beitrage z. Kenntniss des Kehlkopfes u. der Trachea. Wien, 1868, p. 3.

Luschka,²³ after making a study of larynges in which the musculature was well developed, came to the conclusion that the muscle fibers belonging to the free border of the ligamentum vocale pass along the whole length of the ligament, retaining their muscular characteristics from the arytenoid to the thyroid cartilage. His findings in the larynges of children verified his conclusions as to the condition in the adult.

Henle²⁴ states that the fibers of the *musculus thyreo-artyænoideus internus*, adjacent to the ligamentum vocale, are small. The fibers lying nearest to the ligament pass in between the elastic fibers composing it, and are closely connected with them. A number of the muscle fibers either arise from or end among the elastic fibers of the ligament. Regarding the functional significance of this relation, he says that the fibers ending in the elastic tissue must have some influence upon the movements of the ligamentum vocale, and suggests that the short fibers acting upon segments of the ligament may account for the production of falsetto tones.

Jacobson²⁵ describes the *musculus thyreo-arytænoideus* as having a very complicated structure. He finds, in horizontal sections, muscle fibers arising from the processus vocalis, and the lateral surface of the lower part of the arytenoid cartilage, which pass inward to the free border of the ligamentum vocale, and end in bundles of parallel elastic fibers, which eventually pass into the ligamentum vocale. He sums up his conclusions concerning these muscle fibers by saying that there can be no doubt that the *musculus aryvocalis* of Ludwig may be so developed in some cases, that the ligamentum vocale may be rendered tense, while the arytenoid cartilage remains stationary or in the position of adduction. Thus, the short fibers of the muscle may oppose the long fibers, which act as adductors.

Kanthack²⁶ states that the medial fibers of the *musculus thyro-arytænoideus* pass between the elastic fibers and appear to end in them. In sections, which are made exactly parallel to the course of the muscle fibers, it can be seen, however, that they pass uninterruptedly from arytenoid to thyroid cartilage, without ending among the elastic fibers of the ligament. The ligament is not to be regarded as the tendon of the muscle.

Friedrich²⁷ notes that there is no definite arrangement of the elastic

²³ Luschka: *Der Kehlkopf des Menschen*. Tübingen, 1871, p. 121.

²⁴ Henle: *Handb. der Eingeweidelehre des Menschen*; p. 266.

²⁵ Jacobson: *Arch. f. Mikr. Anat.*, Bonn, Bd. xxix, pp. 624-627.

²⁶ Kanthack: *Arch. f. path. Anat.*, etc., Berl., Bd. cxvii, p. 542.

²⁷ Friedrich: *Archiv. f. Laryngol. u. Rhinol.* Bd. iv, p. 207.

fibers about the end of the muscle fiber. He is inclined to believe, however, that there is a close relation between the muscle fibers and the elastic elements of the cord, and emphasizes the fact that by muscle fibers leaving the body of the muscle and running for a short distance in the ligament, as fine an influence could be exerted upon the elastic elements of the ligament as could be explained by the idea of the insertion of muscle fibers directly into the elastic fibers. He does not regard the ligament as the tendon of the *musculus vocalis*.

Katzenstein²⁸ does not regard the *ligamentum vocale* as the tendon of the *musculus vocalis*. He has never seen the direct transition of muscle fiber into elastic fiber.

In horizontal and frontal sections, muscle fibers may be found, which are closely related to the elastic fibers of the *ligamentum vocale*. I have found these to be most numerous posteriorly, in front of the vocal process of the arytenoid cartilage. These muscle fibers have no such complicated arrangement as Jacobson depicts. They seem to pass in between the elastic fibers of the ligament, and to be surrounded by these fibers, but it is probable that they do not end among the elastic fibers of the ligament. Smirnow's investigation²⁹ as to the mode of insertion of striated muscle into soft tissue will aid in settling this question. He says that in all cases in which striated muscle is not in direct relation to the bony or cartilaginous skeleton, in which the fibers are attached to the softer varieties of connective tissues, these tendons consist, wholly or almost wholly, of elastic tissue. In attempting to establish this relation, I have been unable to find in any case a transition of muscle fiber into elastic tissue.

The laryngoscopic findings in the production of falsetto tones as given by Störk, quoted by Jacobson, would suggest that the *ligamentum vocale* may act in segments. I am inclined to believe that these fibers, which are so closely related to the elastic tissue of the *ligamentum vocale*, but still cannot be considered as inserting into it, may by their contraction make tense the vocal ligaments, while the arytenoid cartilage remains stationary, and may by their contraction render the production of falsetto tones possible. There is still another possibility, however. The fibers of the *ligamentum vocale*, as they pass forward to their anterior attachment, are re-inforced by additional elastic fibers, which are derived from the perimysium of the *musculus vocalis*, and through it are attached to the arytenoid cartilage. It is possible that by the contraction of muscle fibers related to these elastic elements different segments of the cord

²⁸ Katzenstein: Arch. f. Laryngol. u. Rhinol. Bd. xiii, p. 346.

²⁹ Smirnow: Anat. Anz., Jena. Bd. xv, p. 488.

could be acted upon, and the cord abducted and rendered tense, while the arytenoid cartilage remained in a position of adduction.

MIDDLE ZONE.

THE ELASTIC TISSUE OF THE VENTRICULUS LARYNGIS.

The continuation upward of the subepithelial elastic layer of the labium vocale forms the delicate elastic membrane surrounding the ventriculus laryngis. This membrane lies just beneath the epithelium of the ventricle, and is poorly developed. Above, it becomes continuous with the elastic tissue occupying the plica ventricularis. Transverse sections through the lower part of the ventricle and through the vocal ligament show well the relation existing between the elastic fibers belonging to each at this level (Fig. 7). The elastic fibers upon the medial surface of the ventricle are continuous with the lateral fibers of the ligament. The elastic fibers upon the lateral surface of the ventricle become continuous posteriorly with the fibers of the ligament, or are attached to the antero-lateral surface of the arytenoid cartilage. Anteriorly, as they approach the nodulus vocalis, they divide, part to be inserted into the nodule and part to be reflected laterad to become continuous with the elastic fibers of the perichondrium, covering the inner surface of the thyroid cartilage.

SUPERIOR ZONE.

ARYTENO-EPIGLOTTIDEAN LIGAMENTS: MEMBRANA QUADRANGULARIS.

The elastic fibers of this zone are situated in the aryteno-epiglottidean folds. Their general direction is from above and anteriorly, downward and backward. Posteriorly, these fibers are attached to the medial surface of the arytenoid cartilages, and anteriorly, to the lateral borders of the epiglottis. Above, they pass upward to the free borders of the folds, and are related to the cartilages of Santorini and Wrisberg. Below, the fibers become thickened to form the ligaments occupying the labia ventricularia.

LIGAMENTUM VENTRICULARE: LIGAMENTUM THYREOARTENOIDEUM SUPERIUS.

Luschka describes the elastic fibers forming the ligamentum ventriculare as being grouped into well-defined bundles anteriorly and posteriorly. In the middle of their course the elastic fibers are separated from each other by numerous glands.

Verson³⁰ denies the existence of a proper ligamentum ventriculare. He states that the elastic fibers occupying the labium ventriculare have no definite direction. A section made at right angles to the labium reveals some elastic fibers which are separated from each other by numerous glands. Interspersed among the elastic fibers are numerous collagenic fibers.

Henle³¹ describes the ligamenta ventricularia as arising on each side of the ligamentum thyreo-epiglotticum from a connective tissue mass, which fills the angle of the thyroid cartilage at this level. Anteriorly, the ligament is an independent band. Posteriorly, the fibers separate to enclose spaces, which lodge glands and fat. In the vicinity of the arytenoid cartilage, between the spina superior and inferior, a band of elastic tissue passes downward posterior to the ventricle. This is the ligamentum arcuatum of Tortual.

Henle's description of this ligament seems to be the most accurate. The elastic fibers arising from the angle of the thyroid cartilage unite to form a distinct ligament in their anterior third. (See Fig. 10.) The ligament is not dense, and the single fibers composing it can be readily recognized. It arises from a connective tissue mass occupying the angle of the thyroid cartilage, which is much smaller than the one at the level of the ligamenta vocalia. In the posterior two-thirds of their course the fibers composing the ligament separate from each other and enclose spaces, in which are lodged numerous glands and fat. The fibers of the ligament anastomose frequently with each other. Numerous collagenic fibers are scattered among the fibers composing the ligament.

The elastic cartilage forming the epiglottis is broken up by numerous glands. (See Fig. 11.) I have attempted to find in this elastic cartilage a definite functional arrangement of the fibers, but this has been impossible because of their number and frequent anastomoses.

The discussion as to the relation existing between the epithelium and elastic tissue of the larynx has been definitely settled by the use of specific stains. The elastic fibers are differently directed in different divisions of the larynx, and bear a different relation to the epithelium in various regions. At the level of the ligamenta vocalia the epithelial cells rest directly upon an elastic fiber layer, which is only arbitrarily separated from the elastic fibers of the ligament. The subepithelial fibers are

³⁰ Verson: Stricker's Handb. der Lehre von den Geweben des Menschen u. der Thiere. Leipzig, 1871, p. 459.

³¹ Henle: Handb. der Eingeweidelehre; p. 254.

parallel to the fibers of the ligament, and to the mucous folds found at this level. The elastic fibers enter the bases of the latter, but do not pass vertically into them. An exception to the latter statement is found upon the medial surface of the vocal process of the arytenoid cartilage, where the fibers of the subepithelial layer pass vertically or obliquely into the base of a small mucous fold. This change in direction of the elastic fibers corresponds approximately to the level at which the linea arcuata inferior of Reinke ²² crosses the vocal process. This line limits the extent of an œdema of the labium vocale posteriorly; the vertical arrangement of the elastic fibers probably acts as a barrier at this point. This arrangement may have some further functional import. In this region the epithelium is subject to the greatest stress, owing to the frequent and wide range of movement of the processus vocalis. The arrangement of the elastic fibers here undoubtedly anchors more securely epithelium to vocal process. Reinke states that occasionally a similar relation between epithelium and subepithelial elastic layer is found at the level of the nodulus vocalis. I have not found this relation in my specimens. In the subglottic region the subepithelial elastic fibers are separated from the epithelium by a thin connective tissue layer. The elastic fibers are here vertically directed. The same relation is found in the inter-arytenoid space, and in the superior laryngeal zone.

I am indebted to Mr. Leonard H. Wilder, artist to the laboratory, for the accompanying drawings.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1.—Frontal section through the anterior part of the conus elasticus. 1. Thyroid cartilage. 2. Elastic fibers of the conus passing mediad on each side to form the ligamentum cricothyreoideum medium, which is pierced by the crico-thyroid vessels. Most of the fibers pass cephalad and laterad from the cricoid cartilage to attach to the thyroid cartilage. Some arise from a raphé formed by the union of the horizontal fibers of the ligament. 3. Cricoid cartilage.

FIG. 2.—Frontal section through larynx, just posterior to the anterior commissure. 1. Thyroid cartilage. 2. Cricoid cartilage. 3. Fibers of the conus elasticus. 4. Nodulus vocalis, showing the convergence of the elastic fibers of the ligamentum vocale upon its medial surface. 5. Musculus vocalis. 6. Ventriculus laryngis. 7. Ligamentum thyreo-epiglotticum. 8. Subepithelial elastic layer separated from the elastic fibers of the conus by glands and collagenic tissue.

FIG. 3.—Transverse section through the ligamentum cricothyreoideum medium, showing its relation to the fibers of the conus and the subepithelial elastic layer.

²² Reinke: Fortschritte der Medicin. 1895, p. 476.

PLATE II.

FIG. 4.—Transverse section through the ligamentum cricothyreoideum medium, showing the general direction of the elastic fibers composing it. Bundles of vertical fibers are surrounded by horizontal fibers, which pass toward the median line to meet corresponding fibers of the opposite side. In this section the crico-thyroid artery is seen piercing the ligament. The direction of the fibers of the subepithelial elastic layer and their relation to the ligament is shown.

FIG. 5.—Transverse section through the larynx at the lower part of the thyroid cartilage. 1. Thyroid cartilage. 2. Conus elasticus. 3. Cricoid cartilage. 4. Posterior crico-arytenoid muscle.

PLATE III.

FIG. 6.—Anterior part of preceding figure as seen under low power, showing the mode of attachment of the elastic fibers of the conus to the thyroid cartilage, and the general direction of the fibers. 1. Thyroid cartilage. 2. Perichondrial process by which the elastic fibers of the conus are attached to the cartilage. 3. Anterior part of the conus elasticus. 4. Subepithelial elastic layer.

FIG. 7.—Transverse section through the larynx at the level of the ligamenta vocalia. 1. Thyroid cartilage. 2. Perichondrial process by which the elastic fibers attach. This is the median process described by Gerhardt. 3. Nodulus vocalis (cartilaginous nodule described by Mayer). 4. Ligamentum vocale. 5. Processus vocalis of the arytenoid cartilage. 6. Arytenoid cartilage. 7. Ventricle of larynx.

PLATE IV.

FIG. 8.—Transverse section through the larynx at the level of the attachment of the anterior extremities of the ligamenta vocalia—low power. 1. Thyroid cartilage. 2. Thickened perichondrium described by Gerhardt as the processus vocalis of the thyroid cartilage. 3. Nodulus vocalis (cartilago sesamoidea anterior); anastomosing bundles of elastic fibers pass off from it anteriorly to be attached to the horizontal fibers of the perichondrium. 4. Parallel elastic fibers of the ligamentum vocale passing into the posterior extremity of the nodulus vocalis.

FIG. 9.—Transverse section through the anterior attachment of the ligamentum vocale. The larynx is divided in the median line. Low power: Van Gieson's picro-fuchsin. 1. Thyroid cartilage. 2. Perichondrial fibers. 3. Nodulus vocalis, showing numerous round and spindle cells. 4. Musculus vocalis.

PLATE V.

FIG. 10. Transverse section through the larynx at the level of the ligamentum ventriculare. 1. Thyroid cartilage. 2. Cricoid cartilage. 3. Ligamentum ventriculare. 4. Fibers of the ligamentum thyreopiglotticum. 5. Ventriculus laryngis. 6. Group of glands.

FIG. 11.—Transverse section through the epiglottis.

THE ELASTIC TISSUE OF THE HUMAN LARYNX

DEAN D. LEWIS

PLATE I

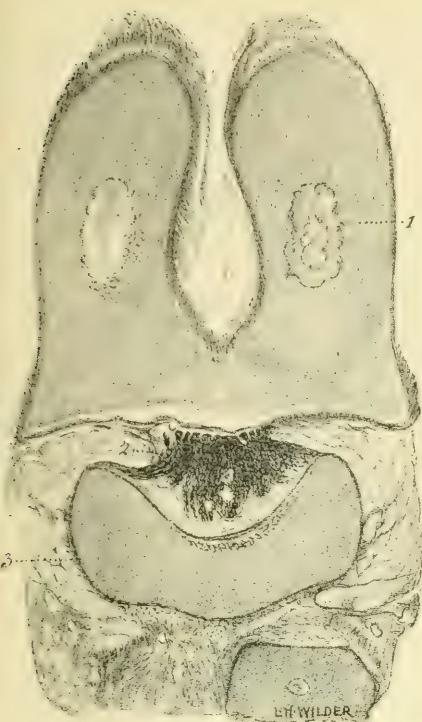


FIG. 1.

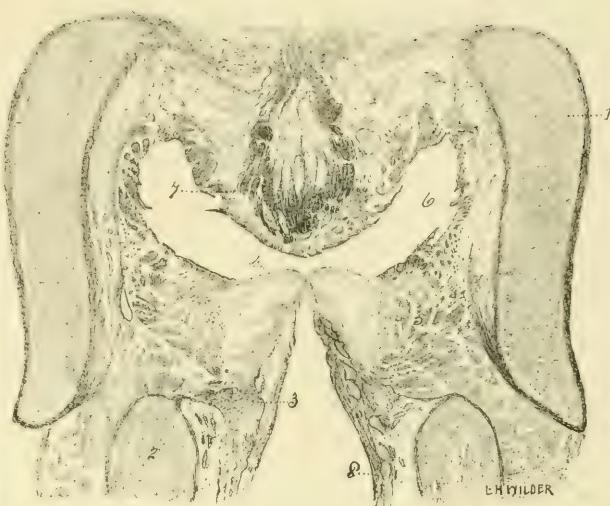


FIG. 2.

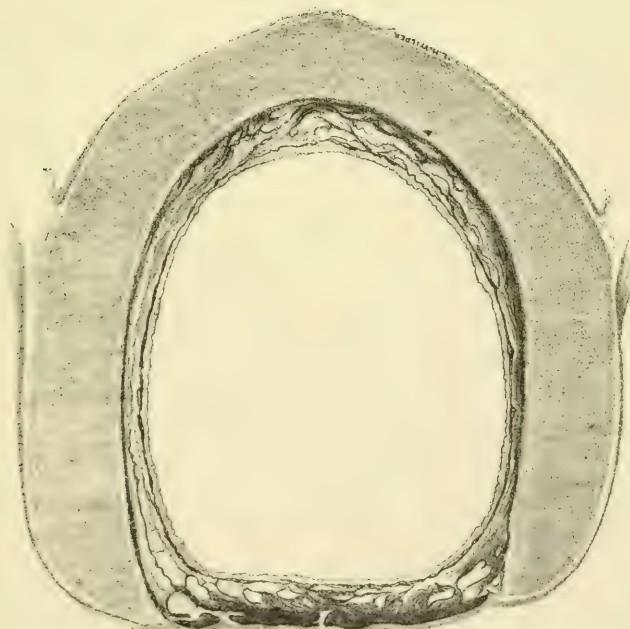


FIG. 3.

THE ELASTIC TISSUE OF THE HUMAN LARYNX

PLATE II

DEAN D. LEWIS

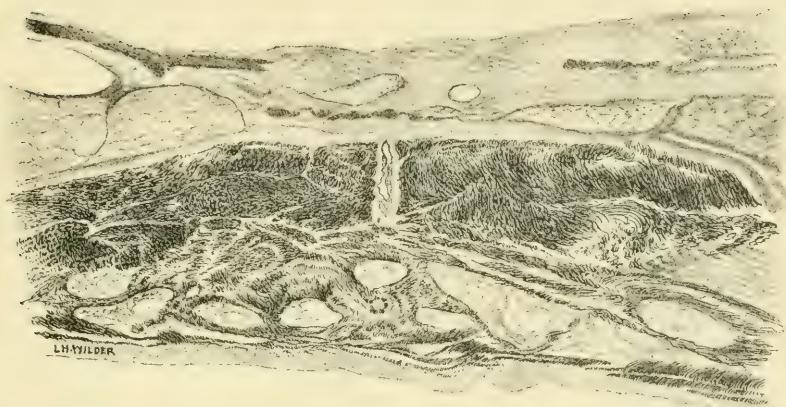


FIG. 4.

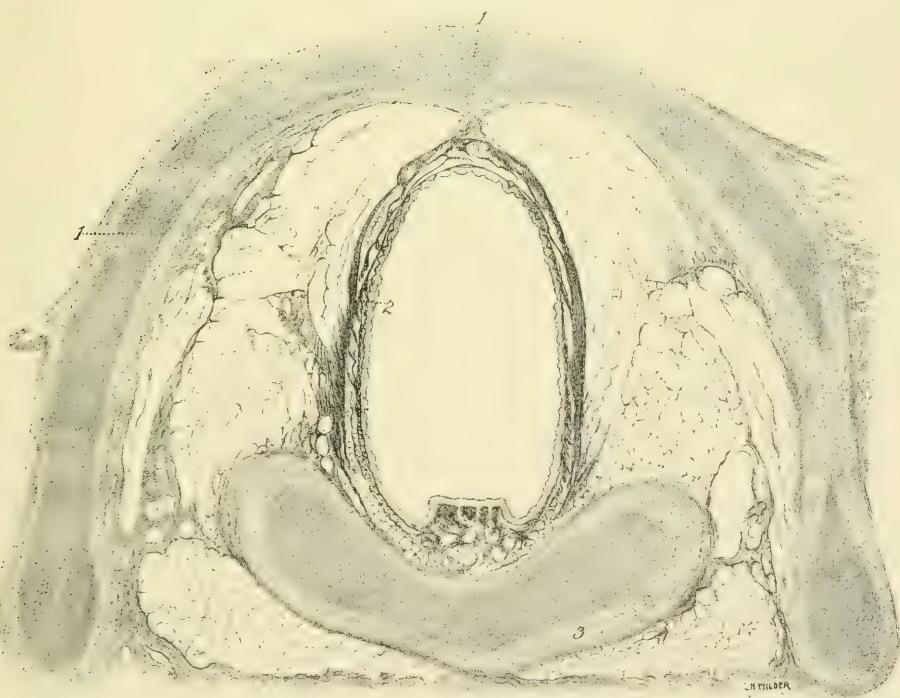


FIG. 5.

THE ELASTIC TISSUE OF THE HUMAN LARYNX

DEAN D. LEWIS

PLATE III

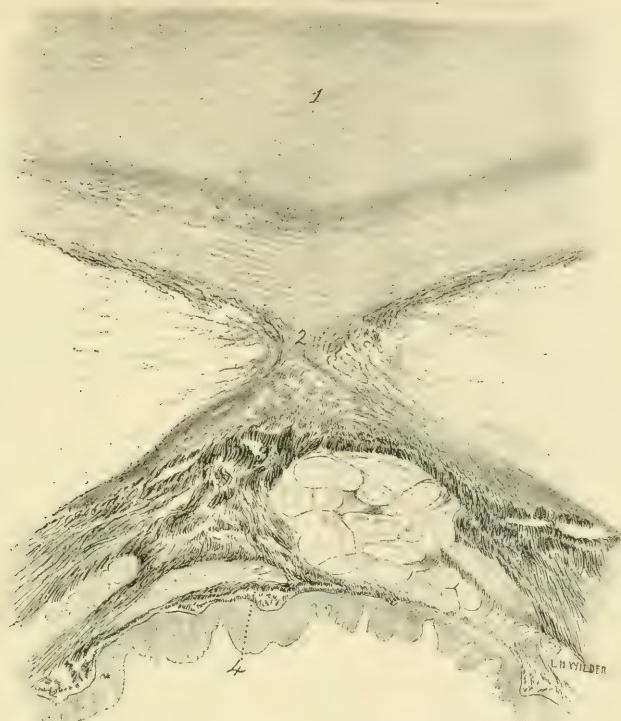


FIG. 6.

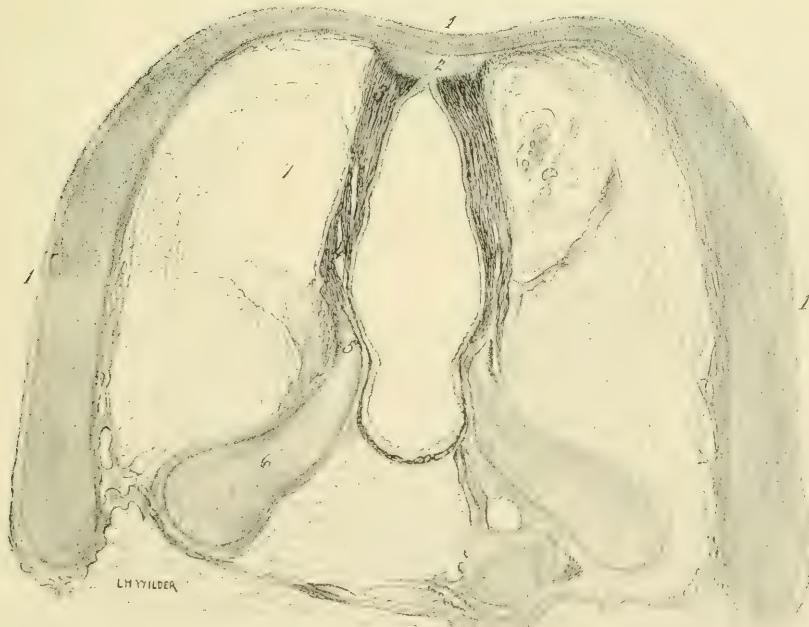


FIG. 7.

THE ELASTIC TISSUE OF THE HUMAN LARYNX

PLATE IV

DEAN D. LEWIS



FIG. 8.



FIG. 9.

THE ELASTIC TISSUE OF THE HUMAN LARYNX

PLATE V

DEAN D. LEWIS

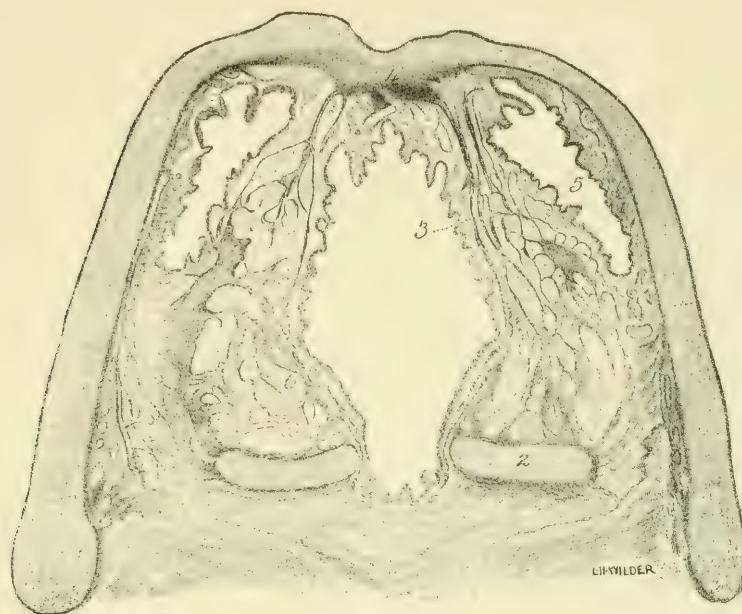


FIG. 10.

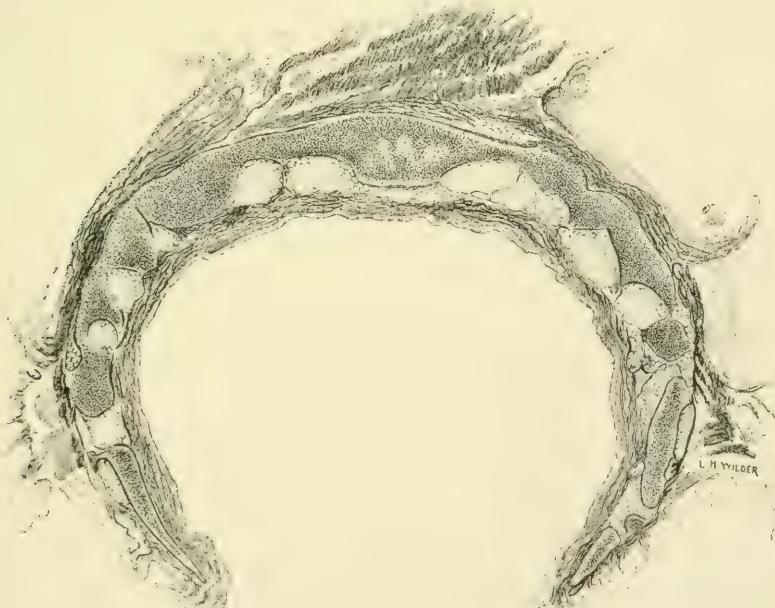


FIG. 11.

STUDIES OF THE INTERSTITIAL CELLS OF LEYDIG.

No. 2.—THEIR POSTEMBRYONIC DEVELOPMENT IN THE PIG.

BY

R. H. WHITEHEAD, M. D.

Medical Department, University of North Carolina.

WITH 5 TEXT FIGURES.

In a recent article¹ I presented the results of a study of the embryonic development of the interstitial cells of Leydig in the pig. In the present article I wish to give a brief account of the findings in a study of their postembryonic development in the same animal. The methods employed were the same as those described in the first article, to which I may refer also for the more important facts in the literature of the subject.

The youngest pig in my series was one month old. In sections of the testis at this age, compared with one from the embryo pig near term, the cross-sections of seminal tubules are somewhat more numerous and closer together, and the masses of interstitial cells are proportionally smaller. Beneath the albuginea the cells are much reduced in size, and are arranged in a few more or less parallel rows, separated by small bundles of connective-tissue fibres. In the deeper portions of the gland, however, the cells are still of about the same size. Three main types of cells can be observed, as follows (Fig. 1) :

1. Cells with cytoplasm condensed around an eccentric nucleus, while the periphery is extensively vacuolated. The vacuoles have more or less uneven, ragged margins. Some of them hold inclusions which vary in size; the largest of these have the granular appearance and staining reactions of the cytoplasm, while others are more hyaline in appearance. Occasionally structures entirely similar to these inclusions are found between the cells.

2. Cells whose cytoplasm is condensed around an eccentric nucleus, while their periphery is much clearer, containing only a few scattered cytoplasmic threads.

¹ Amer. Jour. Anat., Vol. 3, No. 2, 1904.

3. This variety is similar to the preceding, but is characterized by the presence of large acidophile granules, which have about the same size as those noted in the germinal epithelium of the early embryo. They are more granular in appearance, however, than the latter, and in preparations stained by Mallory's method they take the acid fuchsin, whereas the granules of the epithelium are stained by the aniline blue. With Mann's mixture of methyl blue and eosin they are stained by eosin. Although acidophile, they do not stain with as much intensity as the granules of eosinophile leucocytes. The granules are situated, for the most part, in the peripheral portion of the cytoplasm; occasionally a cell is found which seems loaded with them throughout, but, as a general rule, they are largest and most numerous near the periphery. No such granules were seen within the seminal tubules; in the spaces between the Leydig's cells, however, small collections of them were rather frequently encountered, which in most cases were undoubtedly small por-

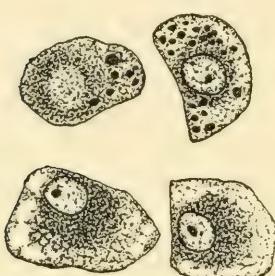


FIG. 1.

FIG. 1. Types of Leydig's cells in pig one month old. $\times 800$.

FIG. 2. Pig three months old. A small group of Leydig's cells. $\times 800$.

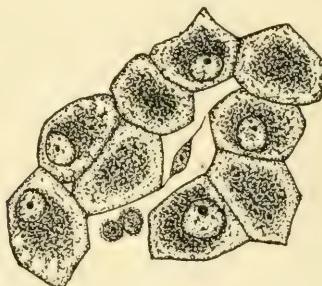


FIG. 2.

tions sectioned from the periphery of granule-bearing cells, but in some instances seemed to be free. All the cells of these three varieties have rather coarse, well defined cell-boundaries, especially marked in the case of the vacuolated cells. These boundaries frequently stain differently from the cytoplasm proper; in preparations stained by Mann's solution of methyl blue and eosin the cell-boundaries quite commonly are blue, while the cytoplasm takes the eosin. Many of the cells have two nuclei, but no mitotic figures were observed. A review of my preparations of the testis from the embryo just before term shows that all these varieties of cells are present there; indeed the principal difference between the two glands, so far as Leydig's cells are concerned, is the atrophy of the subalbugineal layer in the pig one month old. The granules, however,

in the granule-bearing cells of the embryonic gland are smaller and not so limited to the periphery of the cells.

In preparations stained with Sudan III or osmic acid numerous globules of fat, oftentimes very large, are found constantly in the seminal tubules; but the interstitial cells contain at the most only a few fine droplets—many of them contain no fat whatever.

The collections of cells have a rich blood-supply through a network of thin-walled capillaries. A rather striking feature in the testis at this age is the large number of eosinophile leucocytes, both in capillaries and free among the interstitial cells.

In the pig two months old Leydig's cells, in general, are smaller than in the preceding specimen. The varieties of cells described above are still present, but with some differences.

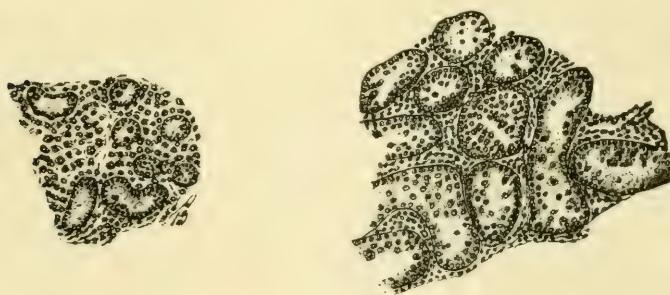


FIG. 3.

FIG. 4.

FIG. 3. A small area of testis in pig one month old. $\times 50$.

FIG. 4. Small area of testis in pig five months old. $\times 50$.

The granule-bearing cells are scanty, while the cells with pale periphery are quite abundant, as are also the vacuolated cells, the two together forming the great majority of the interstitial cells. In the case of the vacuolated cells the number of inclusions is noticeable. In many of these cells the septa between the vacuoles are breaking down.

At three months the convoluting of the seminal tubules has increased considerably, so that many more cross-sections of them are seen, and the interstitial cells are divided up into smaller collections. The breaking down of the septa between vacuoles and the concentration of cytoplasm around the nucleus have also progressed, so that now the individual cells are much smaller than in the preceding stages, and most of them present the pale periphery (Fig. 2). A very few containing acidophile granules may be seen.

These two processes, growth of the tubules and atrophy of the interstitial cells, continue at such a rapid rate that in the five-months pig the tubules greatly predominate over the interstitial cells (compare Figs. 3 and 4). The latter are now so reduced in size as to almost be identical in appearance with the subalbugineal cells of the pig at one month. Individual cells are shown in Fig. 5. Many of them are like the central one in the figure, others entirely lack the distinct cell-boundaries and are little more than naked nuclei, and others show distinct cell-boundaries only at intervals, especially at the margin of a vacuole.

Of the three adult testes at my disposal two were evidently pathological, as the tubules in one case contained no sexual cells, and in the other only a few spermatogonia; probably they were ectopic testes, and need not be considered here. The third one, however, was normal, and spermatogenesis was quite active. Sections show that the growth and convoluting of the seminal tubules have progressed still further, with the result that there are very few Leydig's cells between the albuginea and



FIG. 5. Types of Leydig's cells in pig five months old. $\times 800$.

the bases of the tubules, but they have been crowded against the lines of attachment of the septa to the albuginea. In the deeper portions of the sections the general appearance of the cells is quite similar to that found in the five-months pig, with the exception that they are somewhat larger. They do not contain any acidophile granules, nor could Reinke's crystalloids be demonstrated in them. The subdivision of the groups of Leydig's cells has increased, and in many situations there are none between adjacent tubules.

While, of course, this study does not warrant conclusions as to the function of Leydig's cells in the adult, we may at least inquire if it furnishes any data in support of any of the hypotheses which have been advanced as the result of histological investigation of adult conditions. In favor of the theory of v. Bardeleben, that Leydig's cells replace Sertoli cells as the latter are worn out in the performance of their function, I can find no evidence in any of the preparations. After the basement membrane of the tubules is laid down it forms a barrier which completely prevents the passage of interstitial cells into the tubules. So also as to the view

of Plato, that the function of Leydig's cells is to store up fat and pass it on through the walls of the tubules to be used as pabulum in spermatogenesis, the evidence is negative. The Leydig's cells of the pig's testis contain little or no fat, while the tubules show large quantities of that substance; nor could I detect the minute canals described by him in the walls of the tubules. Moreover, if recent investigations upon fat metabolism are to be accepted, fat entering the tubules from the outside would probably pass through their walls, not as such, but rather as its two liquid components. Some support, however, might be derived for an extension of Plato's theory as suggested by v. Lenhossek, according to which the function of the interstitial cells is to store up, not merely fat, but other material as well, to be used as pabulum by the tubules. The most important facts in the development of Leydig's cells, it seems to me, are the alternating periods of hypertrophy and atrophy, and the structural characters of these cells during the stage of hypertrophy. The periods of hypertrophy precede, while those of atrophy are synchronous with, periods of rapid growth by the seminal tubules. Moreover the changes in the interstitial cells, though occupying much more time, are comparable, to some extent, with those which occur in secreting cells. So that the appearances described might be interpreted as possibly indicating that the Leydig's cells elaborate a specific pabulum for the tubules during the development of the testis.

I wish here to thank Professor F. P. Mall for the courtesy of a seat in his laboratory while this article was in preparation.

PROPHASES AND METAPHASE OF THE FIRST MATURATION SPINDLE OF ALLOLOBOPHORA FÆTIDA.

BY

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WITH 9 PLATES.

In the most mature eggs found in the ovaries of *Allolobophora fætida*, the germinal vesicle is intact, the large nucleolus is present and the chromosomes are not yet formed.

In the eggs of the freshly deposited cocoon, the first maturation spindle is at the metaphase.¹ From the time, therefore, that the eggs leave the ovaries, until they reach the cocoon, the prophases of the first maturation spindle take place, i. e., the forming of the chromosomes, the breaking down of the germinal vesicle, and the disappearance of the large nucleolus. In the summer of 1901, we found that these stages of development occur, while the eggs are in the *receptacula ovorum*² and we have thus far observed no exception to this rule.

In an earlier paper (Foot and Strobell, '02), we demonstrated that these worms deposit their cocoons about every third day, and it is therefore probable that the eggs accumulate in the *receptacula* during this period, indicating that the development of the eggs progresses very slowly in the *receptaculum ovorum*. The earliest stages shown by these eggs, have the germinal vesicle intact, with the nucleoplasm still undifferentiated into distinct chromatic and achromatic substances, a large nucleolus with one or more vacuoles, and no indication of any centriole or rays in the cytoplasm, while the most mature eggs have the first maturation spindle at the metaphase.

¹ We have found only one egg in which the chromosomes are not oriented in the equator of the spindle. The normal stage of development appears to be the metaphase of the first spindle, though there are many degenerated and disintegrated eggs, some showing a vestige of a germinal vesicle but with no differentiation of the nucleoplasm, the chromatin being disintegrated and structureless, and in only one or two cases have we found even an indication of a persisting nucleolus.

² Marshall and Hurst state in their text book, "Practical Zoology" (1888), that immature eggs may be found in the *receptacula ovorum* of *Lumbricus* at certain seasons of the year.

METHOD.

In the summer of 1901, we fixed and sectioned a large number of *receptacula ovorum*, but we found this a very unprofitable method; accurate study of the normal egg being very much hampered by the number of abnormal eggs found in the *receptacula*, in some cases the entire *receptaculum* being filled with eggs in various stages of degeneration. We found in one *receptaculum ovorum* as many as forty eggs and of these only four were normal. A more serious difficulty was the unfavorable action of the fixative on the *receptaculum* as a whole. The swelling or shrinking, or the combination of both, produced by some fixatives, acts with intensified effect on the mass of eggs crowded into the *receptacula*, often distorting the normal eggs in a way to render them valueless for cytological study.

In the summer of 1902, we tried removing the eggs from the *receptacula* after they had been cut from the worm and placed in a watch glass in distilled water. By carefully teasing the walls of a *receptaculum*, under a dissecting microscope, the eggs can be pressed out, and only those that appear normal selected for study, the subsequent technique being the same used for eggs collected from the cocoons (Foot, '98). The following fixatives were used, chromo-acetic, corrosive sublimate, corrosive acetic, Rabl's picro-sublimate, Boveri's picro-acetic, picro-sublimate, Flemming's chromo-aceto-osmiae, and the same proportion of chromic and osmic omitting acetic acid. Hermann's platino-aceto-osmiae and the same proportion of osmic and platinum chloride, omitting the acetic acid. A comparison of the photographs will show that the platino-osmiae has proved the least injurious to both nuclear and cytoplasmic structures. The sections were stained with iron-hæmatoxylin followed by dilute Bismarck brown, or with Bismarck brown alone. In some cases, unstained sections give the most satisfactory photographs. This is especially true for archoplasm, where in stained preparations the dense stain taken by the archoplasm produces such a strong contrast to the faintly stained cytoplasm, that it is impossible to get an accurate reproduction of one without sacrificing the other. All the preparations were stained with the end in view of securing satisfactory photographs, as we aim to present only such cytological phenomena as can be clearly demonstrated by photography.

The impossibility of securing a clear demonstration of the prophases in fixed and sectioned eggs led us to devise a new method which is a modification of the smear method. Instead of smearing a mass of eggs on the slide, we handle each egg separately. After isolating a living egg

in a very small drop of water on the slide, the membrane is carefully pricked with a very fine needle and as the cytoplasm flows out, the egg membrane is gently dragged away with the needle, allowing the contents of the egg to spread and dry immediately. By this method the germinal vesicle, and sometimes even the spindle, flow out of the egg membrane intact, and dry so quickly that the structures are remarkably well preserved. The vesicle when drying flattens out over a larger area, leaving the individual chromosomes or threads sufficiently separated to be clearly identified, and when the entire spindle passes out of the egg membrane intact, all the eleven chromosomes are beautifully demonstrated on one plane. In this manner we arrange from 20 to 30 eggs on a slide, which has been previously ruled with a diamond into definite areas, so that the position of each egg is known and any egg can be studied later in connection with data taken before it was killed.³

Cytoplasm.—During the development of the egg when the prophases of the first maturation spindle occur there is a marked change in the structure of the cytoplasm and during this period there is a decrease in the size of the egg and an increase in the amount of the substance between the egg membrane and the outer membrane, greatly increasing the width of the latter area. Compare the space between the two membranes shown in Photos. 13, Plate I, and 68, Plate IV, eggs at the germinal vesicle stage, and that of Photos. 99 and 100, Plate VI, where the first maturation spindle is at the metaphase.⁴ We have demonstrated these two membranes in earlier papers (1897, Fig. 2) (1901, Photos. 57 and 59), and shown that *Allolobophora* possesses in common with many *Oligochaetes*, a delicate outer membrane and an equally delicate membrane in contact with the egg itself, the space between the two being filled with a relatively non-stainable substance. Vejdovsky and Mrázek have observed these two membranes with coagulated substance between them in many *Lumbricidae* and they criticise Gathy's interpretation of the three layers in *Tubifex* as one thick membrane.

The difference of size and density between the eggs at the germinal vesicle stage and those containing the first maturation spindle is very evident in living eggs, the latter being smaller, more dense and more opaque and these features are equally evident in the dried preparations. At the germinal vesicle stage the typical cytoplasm shows a distinct

³ Such slides do not resemble smear preparations, they suggest rather slides with a few thick sections far removed from one another.

⁴ This contrast is really more marked than shown in the photographs, as Photos. 13 and 68 are magnified nearly 300 diameters more than Photo. 99.

honey-comb structure and at the spindle stage there are fewer alveoli and those present are much smaller. The increase in the amount of substance between the inner and outer membranes of the egg and decrease in the number and size of the alveoli, suggest that the former may be increased at the expense of the latter, but we have no proof of this.

Osmophile Granules.—Typical osmophile granules in the cytoplasm of normal eggs found in the *receptaculum ovorum* are demonstrated in the unstained sections of Photos. 68 and 69, Plate IV. A comparison of these sections with the ovarian eggs of Plate 42 of an earlier paper (1901) shows a diminution in the amount of osmophile substance in these older oöcytes. In the above mentioned paper we noted a decrease in the amount of osmophile substance between the ovarian and the cocoon eggs and suggested that the storing of the osmophile substance in the ovarian egg must be for the use of the egg from the time it leaves the ovary until it is supplied with the nutritive albumen of the cocoon, i. e., during the prophases of the first maturation spindle. This supposition seems confirmed now that we know these processes go on very slowly in the *receptacula ovorum* and that the osmophile substance gradually diminishes in amount during this period.

We first demonstrated these osmophile (deutoplasmic) granules in 1898, and in subsequent papers have presented additional data. We have shown that they are not dissolved out of the cell by either turpentine or xylol, but that after staining they are as a rule invisible, having lost all trace of the blackening caused by the osmic acid. This also fades in unstained eggs that have been kept for a long time in paraffine or mounted in balsam. If a section is photographed before staining, Plate IV, Photos. 68 and 69, and then stained in iron hæmatoxylin the granules are indistinguishable even with a 2 mm. lens, but if the section is compared with a photograph of the unstained preparation that shows exactly where to look for each granule, they can be identified as clear, colorless bodies that would entirely escape observation without the photograph as a guide.

Centriole and Spindle.—In this paper we shall adopt Boveri's term centriole for the small central granule of the asters for which in an earlier paper we retained the old term centrosome. We drop the term centrosome for this granule not because we find a second body in the asters of *Allolobophora* which answers to the centrosome of Boveri and others, but to avoid the confusion of retaining an old term which implies a structure and accompanying complicated changes which we have not found in this egg.

The centrioles destined for the two poles of the first maturation

spindle are first seen at opposite poles of the germinal vesicle, indicating that they arise independently and not by division of a primary centriole. This stage is shown in Photos. 81, 82, 84 and 86, Plate V. The eggs of Photos. 81 and 82 are greatly marred by poor fixation, chromo-acetic being as harmful to cytoplasm as it is to nuclear structure (see p. 215). The centriole of each aster is so small that only the presence of the rays justifies our interpreting the central microsome as the centriole. In Photo. 81, Plate V, the centriole is in contact with the membrane of the germinal vesicle, and in Photo. 82, Plate V, only slightly removed from it. The two asters are at opposite sides of the germinal vesicle, being separated by fifteen sections, the entire germinal vesicle being cut into nineteen sections. In all these sections the membrane of the germinal vesicle is intact and at the points where the rays of the asters focus the membrane slightly protrudes.⁵

It might be claimed that the theory of the nuclear origin of the centriole is supported by the presence of these two centrioles almost within the germinal vesicle, but they certainly do not support the particular phase of the theory that holds the nucleolus responsible for the centriole, for in this egg the nucleolus is still intact and is found two sections removed from one aster and thirteen sections from the other. The injurious action of chromo-acetic on the nucleoplasm of the germinal vesicle does not always affect its membrane, although it breaks the connection between the two, leaving the membrane in contact with the surrounding cytoplasm, this being favorable for the identification of the earliest appearance of the centriole. On the contrary other photographs show that many fixatives favorable for the study of nuclear constituents are of no value for studying the early appearance of the centrioles, the cytoplasm being torn away from the germinal vesicle, destroying all trace of the centrioles and asters (Photos. 22 to 25, 29 and 30, Plate II, 46 to 49, 51 and 52, 59 to 63, and 65, Plate III). In a few cases we have found eggs killed in these same fixatives showing an equal shrinkage of cytoplasm and nucleus leaving the membrane in continuity with cytoplasm and nuclear reticulum, but the exceptions are very rare and have not been at the stage to throw any light on the origin of the centrioles. Platino-osmic, as stated above (p. 200), appears to be the least injurious for all constituents, and we hope its further use will enable us to collect more

⁵The blurred effect of the part of the membrane seen in the photographs is due to its being on a different plane from the centrioles. The two sections shown in Photos. 81 and 82 are cut so close to the periphery of the germinal vesicle that part of its membrane is seen en face. In the sections beyond those photographed, the membrane is the only part of the germinal vesicle left.

satisfactory data as to the origin of the centrioles of the first maturation spindle.

Meves', '02, observations as to the constancy in size of the centrioles, regardless of the size of the cells are supported by this egg. There is an insignificant difference in size between the centrioles at the metaphase of the first maturation spindle (Photos. 26, Plate II, 91, 92a and b, Plate V, 99, 100, 101, 102 and 106, Plate VI), the second maturation spindle (Photos. 103, 104, 105, 108 and 109), the first, second (Photo. 110), and third cleavage spindles (Photo. 107, Plate VI).⁶ There are often exceptions to this rule, but in many of these cases the cause is obviously overstaining (Photo. 83, Plate V), for centrioles that we have seen or photographed in unstained preparations, as a rule, show no more variation in size than can be accounted for by different fixation, or individual variations. Photos. 92a and b, Plate V, show also a similarity in size of the centrioles of the peripheral and inner poles of the first maturation spindle. There is, however, a dissimilarity in size between the centrioles of the prophases and metaphases of the first maturation spindle, the former being smaller, indicating that the centriole passes through stages of growth (compare the centrioles of Photos. 81, 82, 84 and 86, Plate V, with those of the metaphase, Plate VI). That centrioles are sometimes found out of the center of the sphere is probably due to fixation, for if fixation can produce such marked variations of cytoplasmic and nuclear structures as demonstrated in these photographs, it is indeed remarkable that the central position of the centriole is maintained as constantly as we find it in these eggs.

The first maturation spindle can be readily identified in the living egg. In Photos. 125, 128, 129 and 130, Plate IX, we see spindles that have retained their form after the contents of a living egg has been pressed out of its membrane and allowed to dry quickly on a slide. In such preparations we have found no trace of a centriole, but we cannot give much weight to this evidence for in all fixed material, both stained and unstained, a centriole is invariably present at each pole. Unstained centrioles are demonstrated in the spindles of Photos. 91 and 99, Plates V and VI, and stained centrioles in the spindles of Photos. 26, Plate II, 92a and b, Plate V, and Photos. 100 to 110, Plate VI.

Photos. 84 to 89, Plate V, suggest that a large part of the spindle is formed of achromatic nucleoplasm. In Photos. 86 to 89 a part of the membrane of the germinal vesicle is still seen and the nucleoplasm within

⁶The magnification of Photos. 99, 102, 108 and 110 is 710 diameters, and Photos. 26 and 109, 1100 diameters. All the others are 1000 diameters.

this area is unmistakably assuming the form characteristic of spindle fibers. Photos. 81, 82, 83, 84 and 86, Plate V, indicate with equal clearness that the cytoplasm contributes to the polar rays. These photographs (84 and 86) suggest a very close if not causal relation between centriole and spindle and may be called in evidence to support the theory that the spindle is formed under the influence of the centrioles. In these preparations the chromosomes certainly do not influence the form of the spindle, for although they are all massed on one side, yet the spindle remains symmetrical in relation to the centrioles. Even if this massing of the chromosomes in one-half of the spindle is not the normal condition it should produce an abnormal and distorted spindle, if the rays were formed only in relation to the chromosomes. We are forced to conclude, therefore, that the spindle is a life expression of the nucleoplasm and polar cytoplasm, or is formed under the influence of the centrioles.

Nucleolus.—In a comprehensive history of the nucleolus, Montgomery, '98, shows what a bewildering number of conflicting interpretations have accumulated around this structure since it was first figured by Fontana, in 1781, and what little progress has been made in solving the problems of its significance, even its morphology being enveloped in a mass of contradictory evidence. We hope to be able to establish the morphology of the nucleolus of the egg of *Allolobophora fætida* by a careful comparative study of its form after killing in a variety of fixatives, by a study of the living egg, and of the dried germinal vesicles, as obtained by the method described on p. 200. We are convinced that its variety in form can be best appreciated when demonstrated by a number of photographs and with sufficient data for each fixative it may be possible to arrive at a correct decision between the artificial and the normal structures.

The germinal vesicle of *Allolobophora* in common with many other forms contains two distinct kinds of nucleolar formations, and for these we shall adopt Flemming's term, *principal* and *accessory* nucleoli. The former is the relatively large nucleolus which has persisted and increased in size since its first appearance in the smallest oöcyte of the ovary and is peculiarly the nucleolus of the oöcyte first order. The *accessory nucleoli* are first seen in the large ovarian eggs, and the earliest stage at which we find them they are very small and in close proximity to the chromatin (Photos. 9, 11, Plate I, 23, Plate II, and 49, Plate III), and after many fixatives stain like the chromatin. The two structures, the *principal nucleolus* and the *accessory nucleolus*, differ in several respects, as a rule only one of the former is present in a germinal vesicle (Photos.

4, 7, 12, Plate I, 22, 27, 29, 31, Plate II, 49, 50, 58, 60, Plate III, 71, Plate IV), whereas the *accessory nucleoli* vary in number from one to six or more.⁷ The *principal nucleolus* is composed of two substances differing in density and producing, though not invariably, the so-called vacuolated appearance, whereas the *accessory nucleoli* are dense homogeneous bodies and are, as a rule, not vacuolated. The *principal nucleolus* and the *accessory nucleoli* are clearly seen in unstained sections, even the smallest of the *accessory nucleoli* appearing homogeneous and refractive.

In Photos. 23, 29, 39, 40, Plate II, 56, Plate III, both kinds of nucleoli are shown in the same section of a germinal vesicle. Photos. 3 and 4 show two sections of the same germinal vesicle, the former containing the *accessory nucleolus* and the latter the *principal nucleolus*. Photos. 6, 7, 8 and 9 are sections of one germinal vesicle, Photos. 7 and 8 showing the *principal nucleolus* and Photos. 6, 8, 9 showing each one *accessory nucleolus*. After certain fixatives, e. g., platino-osmic, the *principal nucleolus* stains very faintly with iron haematoxylin and can be readily decolored, whereas the *accessory nucleoli* retain the haematoxylin with as much tenacity as do the chromosomes.⁸ The *principal nucleolus* disappears before the first maturation spindle is formed, whereas the *accessory nucleoli* often persist as late as the metaphase of the first maturation spindle. They do not always persist, however, until this period, and when they are present, their position is most inconstant, sometimes being within the area of the spindle, even at the equator, but more often in the cytoplasm at different distances from the spindle. The presence of the persisting nucleoli was noted and figured in an earlier paper (Foot, '94), but at that time we had not recognized them as *accessory nucleoli* but supposed them to be fragments of the large egg nucleolus.

In unstained sections the so-called vacuoles of the *principal nucleolus* are entirely transparent and sharply differentiated from the rest of the nucleolus which is dense and refractive (Photos. 14b, 15 to 21, Plate I, 35, 39, 40, Plate II, and 74, Plate IV). Stained preparations, however,

⁷This applies to sections. In all dried germinal vesicles that appear to be normal, we find as a rule only one *accessory nucleolus*, though we have sometimes found two (Photo. 115, Plate VII), rarely three, and in one or two cases five. Sections of fixed eggs indicate that the single *accessory nucleolus* probably owes its origin to the fusing of several small ones.

⁸That the *principal nucleolus* fails to stain readily at these stages when the *accessory nucleolus* stains very intensely may be due to the disintegration of the former, for at an earlier stage of development the *principal nucleolus* stains intensely.

show that many of the vacuoles contain a substance which after some fixatives can be so densely stained that the entire nucleolus appears homogeneous, these results supporting the observations of investigators who claim that the so-called vacuoles of the nucleolus contain a fluid substance. In dried germinal vesicles we have not been able to demonstrate any vacuoles in the nucleolus, but in the living egg we have sometimes seen a single vacuole in the *principal nucleolus*. In one case the nucleolus at first showed no vacuole, one appearing about five minutes after the egg was under observation and persisting until the egg died. In other eggs the large nucleolus at first contained a vacuole which disappeared in about five minutes, this condition persisting until the egg died. It would seem, therefore, that vacuoles in the *principal nucleolus* of living eggs appear and disappear as maintained by some authors.

Many of Montgomery's figures show the vacuole stained in differential colors, but his interpretation that they "are derived from the small fluid globules which first appear in the nuclear sap" is not supported by *Allolobophora*. Photo. 57, Plate III, might be forced to such an interpretation, but in the light of more than fifty other negative examples it must be interpreted with them as merely another expression of the effect of fixation and dehydration on the more fluid portion of the nucleolus. Even unstained preparations show that the refractive parts of the *principal nucleolus* and its vacuoles represent two substances of very different degrees of density and this must produce an unstable condition which is very readily disturbed by fixatives, and must be, therefore, largely responsible for the great variety of forms seen in fixed material.

The vacuoles in the *principal nucleolus* of *Allolobophora* sometimes appear to be true vacuoles and again they appear to be a thin fluid substance which can be stained so as to completely obliterate the vacuoles (see Photos. 22 and 27, Plate II), which will reappear after decoloring as shown in Photos. 37, 42, Plate II, 49, 53, 54, 58, 60, Plate III, etc., and again the contents of many ring-like nucleoli closely resemble the surrounding nucleoplasm suggesting that it has been artificially forced into the nucleolus. This condition is shown in Photos. 36, 38, 43, 44, 45, Plate II, 50, Plate III, 79, Plate IV, 93, 94, 95, 96, and 98b, Plate V, and many of these resemble the nucleoli figured by Coe, '99, in *Cerebratulus*. In some cases, e. g., Photo. 50, Plate III, it is clearly seen that a substance is massed in the nucleolus at the expense of the surrounding nucleoplasm, and Photo. 98a and b, Plate V, show a distinct break in the nucleolar ring indicating how the fluid portions of the nucleolus and the nucleoplasm may be brought into contact. Many nucleoli which are

not vacuolated, contain small dark specks (see Photos. 5 and 14a) which can be clearly seen and photographed in unstained preparations, for they are quite as black as the osmophile granules (see Photos. 68 and 69, Plate IV, for these granules). They differ from osmophile granules, however, in retaining their original color after staining with iron haematoxylin. Photo. 14a shows them in an unstained, and Photo. 5 in a stained preparation, those of the latter were clearly seen and photographed before the nucleolus was stained. In 1888 Vejdovsky figured and described two nucleoli containing granules in the germinal vesicle of *Rhynchelmis* (Taf III) and Montgomery in '98, figured dark granules in several nucleoli, e. g., Figs. 267 to 269, but they cannot be the same as those shown in our Photos. 5 and 14a, for Montgomery finds by change of focus these dark granules can be transposed into small, clear vacuoles. Photo. 14a demonstrates that this is not the case for *Allolobophora*, in this photograph some of the granules are out of focus, not all being on the same plane, yet none of them appear as vacuoles. Photo. 14b is a nucleolus from the same preparation as 14a and shows some of the vacuoles quite as small as the black granules of 14a, but a change of focus on these small vacuoles does not transpose them into granules. The granules in 14a may represent an early stage of degeneration, a later stage being shown in Photo. 75, Plate IV.

The sixty photographs of nucleoli, shown in our plates, represent forms figured by investigators for widely different material. These photographs show not only the varying forms of the so-called vacuoles, but Photo. 4 shows a nucleolus sharply differentiated into the so-called chromatic and achromatic portions, which can be differentially stained, and have been described under a variety of names. Among later papers, such a differentiation of the nucleolus has been demonstrated in *Helix* by Ancel, '02, and in *Teleosteans* by Stephan, '02. Photos. 20, Plate I, 54, 55, 60, Plate III and 77, Plate IV, show the so-called nucleolulus, or endonucleolus, which Montgomery and others pronounce an artefact.

It is impossible to determine how many of the fantastic forms assumed by the nucleolus of *Allolobophora* are artefacts, but the fact that definite forms appear more or less constantly after certain fixatives creates a well-founded suspicion of every form that cannot be verified by comparison with the living egg.

In *Allolobophora* there appears to be no fundamental difference between the *principal nucleolus* and the *accessory nucleoli*, and may not the individualities of the former be due merely to its adaptation to special needs of the egg during its growth period?

In many points the *accessory nucleolus* corresponds to the nucleoli of

the male and female pronucleus. In the vesicles formed at the telophase of the second maturation spindle⁹ a small dense homogeneous nucleolus is first seen in close proximity to each chromosome (Foot and Strobell, '00). These increase in size by growth and by irregular and inconstant fusing with one another. Thus in the resting female pronucleus we find nucleoli, which like the *accessory nucleoli* are inconstant in size and number, and this inconstancy is true also for the nucleoli of the male pronucleus. One or several of these may persist until the metaphase of the first cleavage spindle and like the *accessory nucleoli* may be in the spindle or in the surrounding cytoplasm. These, like the *accessory nucleoli*, are relatively dense homogeneous structures as compared with the large nucleolus of the oöcyte first order, and these points of agreement suggest the possibility of a closer relationship—may not the *accessory nucleoli* of the germinal vesicle arise in connection with the chromosomes of the first spindle *before* instead of *after* their division? If, as held by a number of investigators, the chromosomes of one division are in some manner related to the nucleolar substance of the following rest stage, may not this be established at an earlier period and the *accessory nucleoli* of the germinal vesicle be a precocious appearance of the nucleoli which are so conspicuously absent between the first and second spindles?—the processes involved in the rest stage occurring before instead of after the first division, the origin and growth of the *accessory nucleolus* being part of them. The second division precociously foreshadowed in the four part chromosomes of the germinal vesicle suggests a precedent for this interpretation.

If our interpretation of the *accessory nucleolus* is correct, a like structure should be present in spermatocytes, and there should be two nucleoli in the spermatocyte first order in all cases where a resting stage is omitted between the first and second division. Such a condition is figured by Vom Rath, '92, in *Gryllotalpha*. His Figs. 10 and 11 show two nucleoli in the spermatocyte first order at the spireme stage in which they are conspicuous also in *Allolobophora*, and it is significant and interesting that the two nucleoli in the spermatocyte are nearly equal in size. See also Schreiner's, '04, Figs. 23 and 24.

⁹The transformation of the chromosomes into vesicles at the telophase of the second maturation spindle was first seen in *Rhynchelmis* and described and figured by Vejdovsky in 1887. Our Photo. 32 shows one of these vesicles in a second maturation spindle and indicates that their formation is not necessarily dependent upon a definite form or position of the chromosomes, as this vesicle is formed before the telophase, and the chromosomes have not assumed the shape they usually show, when at the lower pole of the spindle, prior to the formation of the vesicles (Foot & Strobell, '00, Photo. 24).

We hesitate to interpret the *accessory chromosome* of some authors as the equivalent of the *accessory nucleolus*, but it is a suggestive fact that many investigators have interpreted this structure as a nucleolus and there is a significant disagreement as to whether it divides in the first or second spindles, or in fact whether it divides at all. Our interpretation that the *accessory nucleolus* of *Allolobophora* is the true *nucleolus* of the oöcyte second order supports Wilson's, '96, surmise that the *accessory nucleoli* of egg cells "perhaps correspond to the true nucleoli of tissue cells" (p. 93), though he bases this conclusion on his interpretation that the *principal nucleolus* does not correspond to the "true nucleoli of tissue cells."¹⁰ He mentions two kinds of nucleoli in egg cells, the "principal nucleolus," or net knot, and the "accessory nucleoli," which are of the second (smaller) type, and although they do not agree in their affinity for stains with the *accessory nucleoli* of *Allolobophora* (which at this stage stain with greater intensity than the *principal nucleolus* and retain the color with more tenacity even than the chromosomes), they do agree in other more essential points, i. e., in their relation to the single large nucleolus as to size, number, advent and persistence. In a later edition of "The Cell" ('00), Wilson's conclusions are greatly modified and he states that the *principal* and *accessory nucleoli* "differ widely in staining reactions, but it does not yet clearly appear whether they definitely correspond to the plasmosomes and karyosomes of tissue cells." He further says that the *principal nucleolus* "cannot be directly compared to the net knots or karyosomes of tissue cells," leaving the implication that they resemble the true nucleoli (plasmosomes) of tissue cells, although he adds that in color reaction the *accessory nucleoli* are comparable to these, pp. 127 and 128.

In *Chatopterus*, Mead, '98, figures a ring-shaped nucleolus (Fig. 6) closely resembling those of *Allolobophora*, and although he says nothing of *accessory nucleoli* he has demonstrated in several of his figures two or three nucleoli which appear to answer to the *accessory nucleoli* of *Allolobophora*. He says the nucleolus "breaks up into a number of pieces which remain for a time in the vicinity of the spindle, but gradually degenerate and disappear," p. 196. In *Allolobophora* it is the *accessory nucleoli* which often persist until after the first maturation spindle is formed, the *principal nucleolus* disappearing at an earlier period.

¹⁰ "From its staining-reaction this type of nucleolus appears to correspond, in a chemical sense, not with the 'true nucleoli' of tissue cells, but with the net knots or karyosomes, such as the nucleoli of nerve cells and of many gland cells and epithelial cells," p. 92.

Wheeler in *Myzostoma*, '97, finds that the nucleolus persists in some cases later than the second cleavage but he does not identify any *accessory nucleoli*.

The *accessory nucleolus* of *Allolobophora* probably corresponds to the second nucleolus, Gathy, '00, describes in *Tubifex* as arising independently and disappearing later than the first. Gathy, however, does not interpret the nucleolus he sometimes finds persisting until the metaphase of the first spindle as the above mentioned second nucleolus. His description of the gradual disappearance of the nucleoli without fragmentation is supported by our observations on the *principal nucleolus* of dried germinal vesicles (Photos. 121, 122, 123 and 124, Plate VIII), though after fixatives the nucleolus is sometimes seen breaking up into fragments (Photos. 20, Plate I, 31, 39, 41, Plate II, 54, 55, 57, 67, Plate III, 75, Plate IV and 97, Plate V). Dried preparations clearly demonstrate that the *principal nucleolus* gradually loses its capacity to stain, decreases in size and finally disappears while the membrane of the germinal vesicle is still intact (Photos. 114, Plate VII, 121 to 124, Plate VIII). It does not pass out into the cytoplasm there to degenerate as observed in oöcytes in many other forms. This suggests that its functional value is confined to the nucleus, and if our interpretation is correct that there is no fundamental difference between the *principal nucleolus* and the *accessory nucleolus* we cannot accord any special significance to the fact that the *accessory nucleolus*, unlike the *principal nucleolus*, persists in the cytoplasm before its final disappearance. Its cytoplasmic destiny may be due merely to the fact of its later origin and consequently later disappearance, i. e., after the germinal vesicle has been replaced by the spindle. Dried germinal vesicles indicate that a single *accessory nucleolus* is typical of normal oöcytes. Sections of fixed eggs indicate that the single *accessory nucleolus* owes its origin to the fusing of several smaller ones.

Many authors have recognized a more or less radical difference between the large nucleolus of the germinal vesicles and the nucleoli of the cleavage stages. *Allolobophora* does not show the difference between the two nucleoli that Korschelt, '95, indicates for *Ophryotrocha*. In both Annelids the large nucleolus contributes nothing to the formation of the chromosomes, but in *Ophryotrocha* the cleavage nucleoli "vielleicht ein Theil des vorher im Kernkörper niedergelegten Chromatins dem Kernfaden beigefügt wird." He adds "Was die erwähnten Verschiedenheiten des Verhaltens der nucleolen in den Ei- und Embryonalzellen betrifft so liessen sich diese vielleicht durch die recht verschiedenartige

Ausbildung und Funktion der Kerne in den Beiderlei Zellen erklären," p. 579.

There has been a recent revival of interest in the theory that the chromatin destined for the chromosomes of the first maturation spindle is stored at an earlier stage in one or several nucleoli. In a recent paper Blackman, '03, sums up the weight of authority for this view,¹¹ and to his list of authors may be added recent papers by Goldschmidt, '02, Hartmann, '02, Bryce, '01, and especially Le Brun, '01, '02, whose extensive publications on the maturation stages of *Batrachians* are illustrated by many figures demonstrating this point. The evidence furnished by the egg of *Allolobophora* cannot be interpreted as a support for the above theory. It must unquestionably be classed as supporting the interpretation of the many authors who claim that the chromatin destined for the chromosomes of the spindle is at no time aggregated into a large nucleolus. In *Allolobophora* the chromosomes are formed by a gradual segregation of the chromatin which is dispersed throughout the germinal vesicle, and in order to maintain the theory that the nucleolus is the storehouse of the chromatin there should be a definite and constant relation between the formation of the chromosomes and the breaking down and disappearance of the nucleolus. This, however, is not the case in *Allolobophora*, the two processes do not invariably occur in unison. If the chromosomes have their origin in the nucleolus we should *never* find the chromosomes formed while the nucleolus is intact—before it shows any evidence of breaking down. Photos. 27 and 28, Plate II, 51 and 52, Plate III, and 68 to 73, Plate IV, demonstrate that the chromosomes can be formed in the germinal vesicle without any marked morphological disturbance of the nucleolus, these nucleoli not differing essentially from those of the ovarian eggs and from the nucleoli of those eggs in the *receptaculum ovorum* in which the chromosomes are still unformed. This is demonstrated also in the dried germinal vesicles. In Photos. 111 and 113 to 115, Plate VII, the chromatic spireme is formed and the *principal nucleolus* is still intact, showing no evidence of having contributed to the chromatin of the spireme; and in those cases in which the large nucleolus loses in staining capacity, while the chromosomes increase in staining capacity, the phenomenon is probably due to the normal disintegration of the former, and not to a contribution of its substance to the chromosomes. As a rule the *principal nucleolus* has disappeared, or is in process of disappearing when the

¹¹ "Blachmann, Carnoy, Davidhoff, Hermann, Holl, Sobotta, R. Hertwig, Wilson and others," p. 197.

chromosomes are formed, e. g., Photos. 121 to 124, Plate VIII, but Photos. 116 to 118, Plates VII and VIII demonstrate that the disappearance of the *principal nucleolus* may be retarded until after the complete formation of the chromosomes. Such cases demonstrate that the chromosomes in *Allolobophora* are not formed at the expense of the nucleolus. Korschelt, '95, has reached a like conclusion for the Annelid *Ophryotrocha* and in its morphology the large nucleolus of *Ophryotrocha* is strikingly like the large nucleolus of *Allolobophora* (compare Korschelt's figures of the germinal vesicle 72, 74, 75 and 79 with our Photos. 29, Plate II, 49, 58, Plate III). On this point observations on a number of Annelids are in accord (compare *Myzostoma*, Wheeler's Figs. 3 and 4, '97, *Thalassema*, Griffin's Figs. 3, 6 and 8, '99). In *Batrachians*, Lubosh, '02, has supported his criticism of the nucleolar origin of the chromosomes by a reproduction of many interesting photographs of the nucleoli in Triton eggs. A few of them resemble those of *Allolobophora*, cf. his Photo. 8 with our Photos. 5, 14a, and 75, Plate IV, also his Photos. 5 and 18 with our 20, Plate I, 54 and 55, Plate III.

CHROMATIN, A COMPARATIVE STUDY OF SECTIONS AND OF ENTIRE GERMINAL VESICLES DRIED ON THE SLIDE.

The photographs of the germinal vesicles of Plates I to IV show the average results obtained by sectioning these eggs after killing them in the variety of fixatives given on p. 200.

The photographs of Plates VII, VIII and IX show the average results obtained by drying individual germinal vesicles on the slide, according to the method described on p. 200. A comparison of these two sets of photographs demonstrates the advantage of the latter method, the former proving inadequate almost to the point of being misleading, and it is here evident that we have not yet found a fixative for this egg that shows the development of the chromosomes as clearly as they are demonstrated in dried preparations.

In sections, the chromatin of the most mature eggs of the ovary and of the youngest eggs of the *receptacula ovorum*, is at about the same stage of development, the relatively achromatic reticulum of the germinal vesicle showing only indefinite chromatic aggregations which appear to be the first steps towards forming the filaments out of which the eleven bivalent chromosomes are finally formed. The first indications of chromatic filaments are shown in Photos. 1 to 9, and 31, Plate II, and 66, Plate III.

In dried germinal vesicles an early stage of the aggregation of chro-

matin is shown in Photo. 111, Plate VII, where the germinal vesicle is traversed by an extremely delicate chromatic thread or threads. Prior to this stage we have found no structure in the germinal vesicles other than the nucleoli, the entire nucleoplasm being uniformly chromatic and obscuring all differentiation. The only indication of the presence of the diffused chromatin being expressed by the fact that the nucleoplasm reacts to nuclear stains with much more intensity than it does after its chromatin constituent has formed the spireme or chromosomes.

This is true of eggs taken from the free end of the ovary, and the earliest stages of those found in the *receptacula*. A comparison of results obtained from dried germinal vesicles with sections creates the suspicion that much of the structure seen in sections of these eggs in the earlier stage may be due to fixation. Such artificial demonstration of the chromatin is, however, instructive in showing how the *accessory nucleoli* arise in close connection with the chromatin in widely separated areas of the germinal vesicles, later fusing into the one *accessory nucleolus* typical of later stages.

In sections, a more marked aggregation of the chromatin into pronounced chromatic filaments is shown in Photos. 22 to 25, Platé II, 46 to 49 and 59 to 62, Plate III, these filaments undoubtedly corresponding to portions of the skeins shown in the dried germinal vesicles of Photos. 113 to 115, Plate VII.

At the upper, right-hand periphery of the germinal vesicle of Photo. 30, Plate II, there are two isolated, loosely granular filaments, the granules perhaps representing individual chromomeres, which are obscured in the more dense chromosomes of Photos. 28, Plate II, 51, 52 and 64, Plate III. Later stages of chromosome formations are shown in Photos. 68 to 72, Plate IV, and further stages of development in Photos. 85 to 89, Plate V.

In dried germinal vesicles we see a much more intelligible evolution of the chromosomes,—in Photos. 113 to 115, Plate VII, the delicate chromatic thread or threads of Photo. 111 have contracted or fused into a relatively thick spireme. This spireme divides transversely into bivalent chromosomes, the character of each chromosome being clearly expressed by transverse constrictions in the center, demonstrating that each bivalent chromosome is composed of two equal parts. Photos. 114 and 115, Plate VII, show a distinct longitudinal split of the spireme, and Photos. 116, 117, 119 to 130, Plates VII to IX, show the persistence of this split to the chromosome stage, producing typical tetrads.

In all our sections showing the stages represented in Photos. 68 to 70,

Plate IV, 86 to 89, Plate V, we find the chromosomes in a tangled condition, and we are unable to identify in such confused masses of chromosomes the rings or other forms of an earlier stage (Photos. 51 and 52, Plate III). Presumably the small ring of Photo. 52 answers to the small four part chromosome of Photo. 72, Plate IV, and perhaps to the small chromosome of Photos. 33, Plate II, 91, Plate V, and 99, Plate VI, but our sections give no proof of this, chromosomes being so rarely isolated in fixed material that no trustworthy comparison of progressive stages can be made.

In dried germinal vesicles, however, the chromosomes of each stage are clearly isolated and can be readily compared and a few of the chromosomes of the first maturation spindle can be identified in the prophases, though their individual differences are not sufficiently marked to make such identification conclusive.

In the majority of cases the photographs of sections show that the chromatic filaments are partly formed, more or less at the expense of the reticulum in which they are imbedded (Photos. 22 to 25, Plate II, 46 to 49 and 59 to 62, Plate III). We are convinced that this is not comparable to the living condition; but due to fixation, for its degree varies with different fixatives. Eggs fixed in chromo-acetic show the extent to which this process can be carried, the achromatic substance¹² and chromatin being coagulated into thick, loose coils that are so dense they hold the stains with as much tenacity as the chromosomes (see Photo. 34, Plate II). This photograph also shows the nuclear membrane distorted and torn, whereas the membrane of the germinal vesicles of eggs killed in platino-osmic remains unbroken and in perfect contact with the nucleoplasm as well as the cytoplasm. Compare for example, Photo. 34, Plate II, with Photos. 68 and 69, Plate IV, the latter egg showing the smooth contour of the membrane typical of a living egg, and this is also shown in germinal vesicles dried on the slide.

All the sections of germinal vesicles are photographed at the same magnification (1000) and a comparison of any of them with Photo. 34, Plate II, demonstrates the swelling of the germinal vesicles in the chromo-acetic preparation. Watching the effect of chromo-acetic on the living egg under the microscope shows that this fixative first swells the egg before the usual shrinkage of dehydration commences—the final shrinkage being less than that of many other fixatives. The actual size

¹² We use the expression achromatic substance because it is so well established as a definite part of the nucleoplasm as distinct from chromatin. It is often, however, a misnomer, for in some cases it stains intensely (Photos. 27 and 28, etc.).

of the germinal vesicle of Photo. 34 more nearly approaches that of the living egg; but the distortion of the nuclear constituents indicates the injurious effects produced by the chromo-acetic, probably due largely to the initial swelling. Such definite and varying response to fixatives serves to support the present skeptical attitude towards all cellular structure seen in fixed material. The reticulum which must represent the residuum of the achromatic substance after its dehydration, presents a varied aspect, definite fixatives being responsible for definite forms. These form differences are clearly seen by a comparison of the reticulum in Photos. 68 to 73, Plate IV (fixed in platino-osmio) with those of Photos. 51 and 52, Plate III, showing an egg of nearly the same stage of development, but fixed in corrosive sublimate. The achromatic substance of the former (Photos. 68 to 73, Plate IV), is a relatively transparent homogeneous substance in which the chromosomes are imbedded, while that of Photos. 51 and 52, Plate III, is a distinct network. The characteristics of the achromatin of the first egg (Photos. 68 to 73) are as pronounced in the *unstained* sections (Photos. 68 and 69) as in those stained with iron haematoxylin (Photos. 70 to 73, Plate IV), and this egg shows that dehydration and shrinkage have taken place with much less distortion of the nuclear and cytoplasmic elements. The nuclear membrane is intact and the cytoplasm is not torn away from the nucleus as is the case in corrosive sublimate preparations, and in all these eggs killed in fixatives containing acetic acid¹³—compare those of Plates II and III with Plates I and IV. The extent to which a fixative may distort the nucleoplasm is seen in Photo. 34.

The photographs demonstrate that apart from the nucleoli only two constituents are clearly differentiated in the germinal vesicle, the relatively achromatic substance and the sharply chromatic filaments, which, as stated above, appear to be not pure chromatin but chromatin plus a part of the achromatic substance. Photos. 22 to 25, Plate II, 46, 47 and 59 to 62, Plate III, show in many cases clear areas around the filament indicating that the chromatic filaments are partly formed at the expense of the surrounding reticulum, and the fact that after some fixatives, (e. g., chromo-acetic, Photo. 34, Plate II); all the achromatic substance and chromatin are welded together, suggest that the fixative may be responsible even in those cases where only a small part of the achromatic substance contributes to the chromatic filament. This has a distinct bearing on the theory that only a small part of the chromatin of

¹³ Tellyesniczky's, '02, criticism of the use of acetic acid in the study of nuclear constituents is supported by its effect on the egg of *Allolobophora*. It unquestionably produces artefacts in both nucleolus and nucleoplasm.

the germinal vesicle takes part in forming the chromosomes of the first spindle. The evidence points rather to the conclusion that the apparent surplus of chromatin in this egg is due to its earlier artificial combination with the achromatic substance causing a misconception as to the actual amount, and that all the chromatin, excepting that possibly contributed to the *accessory nucleoli*, is finally consigned to the chromosomes.

We have not attempted to analyze the reticulum by differential anilin staining, because the experimenting we have done with anilins on later stages of the egg, has convinced us of the justice of the criticism of those investigators who question all results obtained by this method. Our aim is to place in evidence only such phenomena as can be seen and photographed without the aid of any complicated method of staining, and in nearly all cases we control the evidence of the stained preparations by photographs of unstained sections, e. g., Photos. 68 and 69, Plate IV, and forty other unstained sections showing nucleoli, centrosomes, archoplasm, etc.

With thin unstained sections much can be seen and photographed at a thousand diameters—the centriole and even individual microsomes can be clearly registered by photographs and such evidence as this method furnishes is at least relatively reliable. It may be an objection that this simple method throws out of court a number of so-called nuclear structures, for we are indebted to the anilin stains for several analytical subdivisions of the reticulum, e. g., Heidenhains' lanthanin or oxychromatin granules which, according to Tellyesniczky, '02, are the same as Schwartz's paralinin and Pflitzner's parachromatin—the non-staining linin, and Reinke's oedematin spheres, or cyanophilous granules. It may be justly asked, whether this is a question of indebtedness to the anilins or a score to settle.

Chromosomes.—The development of the 11 tetrads and their subsequent division in the first maturation spindle are so clearly demonstrated by our new method described on p. 200 that the successive steps of the process can be illustrated by a few photographs¹⁴ of these preparations (Plates VII, VIII and IX).

In the dried germinal vesicles of eggs from the distal end of the ovary and of the youngest eggs from the *receptacula ovarum* we have been unable to identify any differentiation of the nucleoplasm into the rela-

¹⁴ We have more than two hundred preparations demonstrating these stages with equal clearness and many of these we have already photographed. In a future paper we shall reproduce some of them in connection with photographs of later stages demonstrated by the same method.

tively achromatic and chromatic segregation which appears later. Photo. 111, Plate VII, shows an early stage of the segregation of the diffused chromatin into a delicate thread or threads which later form the pronounced spireme of Photo. 113. At this early stage of the spireme the entire germinal vesicle is traversed by a delicate thread so closely entwined that it gives the appearance of a network and it has been impossible to determine whether this is composed of one continuous thread. Photo. 111 represents a typical distribution of the chromatin at this stage. We have a large number of similar preparations and many photographs, but lack of space prevents our reproducing more than one.

Photo. 112, Plate VII, shows a very different segregation of the chromatin, the chromatic granules of the nucleoplasm are collecting directly into a coil-like structure without passing through the stage shown in Photo. 111. We have only one such preparation and we interpret it as abnormal, but have reproduced it because it shows so clearly that the chromatin is distributed throughout the entire germinal vesicle, and because that part of the nucleoplasm which is not yet differentiated into chromatin and achromatin gives a very faithful picture of the entire nucleoplasm of oöcytes in an earlier stage of development. The granular nucleoplasm as shown on the right periphery of the germinal vesicle of Photo. 112 gradually segregates (in normal eggs) into an extremely delicate chromatic network, which is at first as indistinct as that shown at the left side of the germinal vesicle of Photo. 111.

Photo. 113, Plate VII, is a germinal vesicle showing a typical early stage of the spireme. A study of this photograph in the light of Photo. 111 suggests that the spireme of Photo. 113 has been formed by a contraction and thickening of the delicate thread or threads of Photo. 111 or by the fusing of parallel strands.

A study of Photo. 114 in the light of Photo. 113, Plate VII, suggests that each part of the double thread of Photo. 114 may be the single thread of Photo. 113, or, as we are inclined to think, that the single thread has increased in thickness by contraction and growth and has subsequently split. The longitudinal split of the spireme seen in Photos. 114 and 115 persists throughout the prophase and can be clearly seen in many of the chromosomes at the metaphase (cf. Plates VIII and IX). We interpret Photos. 114 and 115 as a later stage than Photo. 113 because the thread has commenced to break apart transversely to form the eleven bivalent chromosomes.

In Photo. 116, Plate VII, the entire spireme has divided into bivalent chromosomes with the exception of the two bivalent chromosomes which are close to the *accessory nucleolus*. These are still attached end to end,

forming a part of the original coil including *four* univalent chromosomes or two bivalent chromosomes. The bivalent character is shown in the lower of the two chromosomes by a clear space in the center, and a similar clear space is shown in the bivalent chromosome just northeast of the *accessory nucleolus*.

We have several intermediate stages between Photos. 115 and 116, Plate VII, where the chromosomes are in the form of long thread-like loops, but lack of space prevents our reproducing them. The bivalent character of the chromosomes is clearly shown in many of the photographs. The three rings in the upper part of Photo. 117, Plate VIII, show not only that each ring is composed of two univalent chromosomes attached end to end, but the longitudinal split of each is indicated, completing the transverse and longitudinal markings typical of the tetrad. We may say that the chromosomes of the prophase and metaphase are typical tetrads, for in every preparation in which the eleven chromosomes are shown, one or more of them show beyond question both the longitudinal and transverse markings.

In Photo. 118, Plate VIII, at least five of the eleven chromosomes show the transverse constriction, though in all these chromosomes the longitudinal split is obscured. In Photo. 119 the tetrad character of at least five of the chromosomes is almost schematically shown, the large figure 8 shows not only the longitudinal split but a marked constriction in each loop indicating the point of contact of the two univalent chromosomes. The small chromosome southwest of the figure 8 shows with equal clearness its bivalent character and the longitudinal split, and the three bivalent chromosomes north of the figure 8 admit of only one interpretation. The fact that *eleven bivalent chromosomes* are typical of the prophases of the first maturation spindle of *Allolobophora* is demonstrated by the photographs of Plate VIII and the tetrad character of these chromosomes is clearly shown. A good deal of scepticism has recently been expressed as to the constancy of the number of the chromosomes in the first spindle, discrediting the great significance that has been attached to this point. We would therefore accentuate the fact that in every case where the chromosomes are so distributed as to admit of an accurate count, *we have not found a single exception to the number eleven* in the prophases and metaphase. Rods, rings and figures 8 are the most common forms, though there are examples of the cross-shaped chromosome which several investigators have demonstrated in other forms. In *Allolobophora* an interpretation of their origin appears to present no difficulties, they undoubtedly arise by a simple contraction of a bivalent chromosome, i. e., two rod-shaped univalent chromosomes

placed end to end. As they contract and are pressed together each splits open along the line of the longitudinal furrow, the ends are thus pressed out at right angles forming the two arms of the cross. As our preparations show the cross type of chromosome in all stages of its development, no other explanation of its origin for this egg seems possible. The beginning of the formation of a cross is seen in Photo. 124, Plate VIII, in contact with the asymmetrical figure 8, and northeast of it a cross in a further stage of development. Varying forms of the cross chromosomes are seen in Photos. 117, 120, 123, Plate VIII, and 116, Plate VII. The last photograph shows also the first stage of a cross formation in the bivalent small chromosome at the lower periphery of the germinal vesicle, and in Photo. 126, Plate IX, the method of forming a cross is almost schematically shown in the fourth chromosome from the left periphery of the photograph.

In the preparations reproduced on Plate IX the membrane of the germinal vesicle as well as the *principal* and *accessory nucleoli* have disappeared. The eleven bivalent chromosomes in all cases are present and in Photos. 129 and 130 are symmetrically arranged in the equator of the spindle ready to divide. These preparations appear to us to demonstrate conclusively that the first division separates two univalent chromosomes, but we do not yet know that these two univalent chromosomes are two of the *somatic* chromosomes of the oögonia, so we cannot assert that the first division is a reducing division in Weismann's sense. We can only say that the prophases and metaphase of the first maturation spindle of *Allolobophora* support the observations of Korschelt, Montgomery and others, who do claim that the first division is reducing. But in *Allolobophora* several questions still remain unanswered. Does each bivalent chromosome represent two somatic chromosomes which are exactly similar in size and form, or does this exact similarity only indicate a foreshadowing of the first division? Do the two represent the paternal and maternal inheritance as held by Montgomery, 'or, Sutton, 'or, and others, or does the longitudinal furrow indicate this double line of inheritance? We must delay an attempt to answer these questions until we can determine whether the pairs of chromosomes, represented by the bivalent chromosomes of the prophase, are present in the oögonia as Montgomery and Sutton find them in certain insects, and whether the longitudinal furrow of the prophase can be explained as a foreshadowing of the second division.

The photographs of Plates VII and VIII demonstrate that the ring chromosomes are formed by the uniting of the free ends of two *univalent chromosomes* and the photographs of Plate IX show that such rings are divided at the metaphase at the points of contact of these two chromo-

somes. In most cases this point of contact is expressed by a clear space or by a knob-like thickening. The clear space is shown in the three rings of Photo. 117, Plate VIII, and in one or more of the chromosomes in Photos. 116 to 130, Plates VII, VIII and IX. The knob-like thickening at the point of contact of two univalent chromosomes is shown in one of the chromosomes of Photos. 116 and 130.

That the spindles must have some tenacity of form in the living egg is demonstrated by the characteristic spindle formation with the two polar spheres often remaining undisturbed by the process of pricking the membrane of the egg and allowing the cytoplasm to flow out upon the slide. An indication of the spindle form is shown in Photos. 125, 128, 129 and 130, Plate IX. The fact that the egg is dried so rapidly that the form of the spindle is not distorted argues that some confidence may be placed in the form of the chromosomes as well.

In the equator of the spindle of Photo. 125, Plate IX, a ring chromosome is seen showing a distinct longitudinal split and the clear transverse space which indicates one point of contact of the two univalent chromosomes which form the ring. This space is in the equator and unquestionably indicates one of the points of separation of this chromosome. The chromosome on the extreme left shows a like clear space, the other half of the ring, having already separated and contracted, resulting in one of the forms typical of the metaphase (the lower arm of this chromosome is in contact with the upper arm of a like chromosome). This form of division is seen in two of the chromosomes of Photo. 128, in at least five of the chromosomes of Photo. 129 and five of Photo. 130, Plate IX. Rings with a longitudinal furrow and characteristic indications at the points of contact of the univalent chromosomes of which they are formed are shown in Photo. 126, Plate IX, and three similar rings dividing transversely are shown in Photo. 127, the one near the center of the photograph being especially instructive. These examples, with the ring of Photo. 129 and the two rings of Photo. 130, Plate IX, appear to dispel all doubt as to the manner in which the bivalent chromosome of *Allobophora* is divided in the first maturation division. In each of these six photographs there are examples also of the simple transverse separation of the two rods attached end to end which represent the simplest form of these bivalent chromosomes. Many of them still show the longitudinal furrow which has persisted from the spireme stage and leave no doubt that this division is not along the lines of this longitudinal split.

Many of the chromosomes demonstrated in the spindles of our Plate IX closely resemble those figured by Nekrassoff, '03, in the first matur-

ation spindle of *Cymbulia*, Fig. 7, though he interprets their division as longitudinal.

This egg agrees with the observations of the many investigators who have demonstrated for both oöcytes and spermatocytes a marked difference in the size of the chromosomes of the first spindle. In *Crepidula*, Conklin, '02, has shown this inequality to have reached the ratio of one to fifteen in volume. In *Allolobophora* the inequality in the size of the chromosomes is distinctly seen at the prophases and metaphase; compare the two chromosomes in the same germinal vesicle of Photos. 51 and 52, Plate III, and chromosomes in the germinal vesicle of Photo. 116, Plate VII, and those of Plate VIII. Compare the size of the chromosomes in the first spindle (metaphase) Photo. 33, Plate II, and those of Plate IX.

It is more difficult, however, to demonstrate a persistent and individual form for each chromosome and in fixed and sectioned eggs we have found this quite impossible. For example, in a collection of thirty-four photographs showing every chromosome in four first spindles at the metaphase it was impossible to identify any one chromosome in the four spindles as the same individual. This is undoubtedly due in part to distortion of normal forms by fixation and as a rule the chromosomes are so closely massed in fixed material that their individuality is obscured (e. g., Photos. 69 and 70, Plate IV, 85 to 89, Plate V). In dried germinal vesicles this massing of the chromosomes is avoided and individuals can be distinctly differentiated. Although the individuality of these chromosomes is not sufficiently pronounced to admit of a definite identification of the individual at each stage, a comparison of the chromosomes of the prophases of Plate VIII with those of the metaphase on Plate IX will demonstrate that a few of the individual chromosomes of the prophases can be identified in the metaphase with some accuracy, and this argues strongly for the individuality of all, and supports the theory which has been so frequently and ably defended by Boveri.¹⁵

The prophases of the first spindle of *Allolobophora* as above demonstrated confirm Vom Rath's interpretation of the prophases of the first spindle of the spermatocytes of *Gryllotalpha* published in 1892. When

¹⁵ After our paper had gone to press Baumgartner's interesting article appeared, giving "Some new Evidence for the Individuality of the Chromosomes," Bio. Bull., Vol. VIII, 1904. Our Photos. 116 to 130, Plates VII, VIII and IX demonstrate that Baumgartner's suggestive conclusions are not supported by the egg of *Allolobophora*. We find no constant form differences of the chromosomes, the simplest form of the bivalent chromosome is two rods attached end to end, and these present a variety of shapes, rings, figures 8, crosses, etc., without any regularity or constancy. The free ends of the

the "Samenmuttenzelle" (spermatocytes 1st order of authors) has attained its growth the chromatin is distributed as a delicate "Maschenwerk" (cf. his Figs. 10 and 11 with our Photo. 111, Plate VII). He next figures and describes the chromatin as a coil with a single longitudinal split (Fig. 12), this coil dividing transversely into half the number of somatic chromosomes, each of the bivalent segments representing two somatic chromosomes attached end to end, later their free ends uniting to form rings, these rings showing the same longitudinal split which he demonstrated in the coil. He is uncertain, however, whether this longitudinal split foreshadows the first or second division. He says: "Es kann folglich die eine der beiden Trennungen der Chromosomen auf diese vorseitige Spaltung des Chromatinfadens zurückgeführt werden; ob dies nun aber die erste oder die zweite Theilung ist, kann nach den Präparaten nicht mit Sicherheit entschieden werden, ich möchte eher an die zweite Theilung denken," p. 113.

Montgomery's, '01, '04, interpretation of the prophases studied in a variety of forms, is supported by *Allolobophora* in the longitudinal split of the coil (cf. Montgomery's '04, Figs. 10 and 11 with our Photos. 114 and 115, Plate VII), and the separation of the univalent chromosomes at the first division, but this egg does not support Montgomery in certain points in which his observations differ from those of Vom Rath, Rückert and Häcker. These points are clearly stated by Montgomery, '04: "Rückert, '94a, and Häcker and others after him, concluded that there was a continuous chromatin spireme preceding the first maturation mitosis, and that the apparent reduction in number of the chromosomes is effected by this chromatin spireme segmenting into half the normal number of chromosomes. I showed for *Peripatus*, '00, on the contrary, that a continuous linin spireme is present at this stage but not a continuous chromatin spireme, and that the bivalent chromosomes are produced by a later conjugation without the formation of a continuous chromatin loop. According to Rückert it is a case of chromosomes already closely connected remaining so; according to me, of chromosomes not in contact at first, becoming so secondarily. Hence I spoke of this act as the conjugation of the chromosomes, and argued that this is the important

bivalent chromosomes show a tendency to unite into a ring and in some cases nearly all the eleven chromosomes are rings (Photo. 122), and sometimes not a single ring is formed, Photos. 116 and 118. This by no means disproves Baumgartner's conclusions, for the variety of shapes of the chromosomes of *Allolobophora* may be due to mechanical disturbance of the living form incident to the technique. This point can be determined only by the study of living chromosomes.

criterion of the synapsis stage." The photographs of our Plates VII and VIII demonstrate that in *Allolobophora* "it is a case of chromosomes already closely connected remaining so."

Many of our photographs confirm A. and K. E. Schreiner's, '04, observations on the spermatocytes of *Myxine glutinosa* and *Spinax niger*. The delicate thread-like reticulum in the early prophase (cf. Fig. 2 with our Photo. 111), the coarse spireme of the later stage (cf. their Figs. 3, 5 and 23 with our Photos. 114 and 115, Plate VII), and finally the form of the bivalent chromosomes. The rings, figures 8, etc., of Schreiner's Fig. 15 are reproduced in *Allolobophora*, though in their mode of formation and subsequent division there are fundamental differences. They interpret the first furrow of the spireme as due to a union of two of the delicate threads of the earlier stage, and at a later stage they identify a second longitudinal split of the spireme, these two longitudinal divisions indicating the method of separation of the chromosomes for the first and second divisions. The individuals of each bivalent chromosome are *paired longitudinally in the spireme*, whereas in *Allolobophora* they are placed end to end, thus though the rings, figures 8, etc., of Schreiner's Fig. 15 and those of *Allolobophora* are formed alike by the uniting of the ends of two univalent chromosomes, they have attained this final arrangement by an entirely different method. In both forms, therefore, the first division separates univalent chromosomes, though in one case the division is longitudinal and in the other transverse.

This transverse division of the chromosomes supports Lillie's, '01, observations on *Unio*. He says, "The first division is certainly at right angles to the long axis of the chromosomes, as these lie in the equatorial plate," p. 236.

The spireme demonstrated in our photographs of Plate VII and the "heterotypic" chromosomes of Plate IX confirm Flemming's observations on the spermatocytes of *Salamandra maculosa* published in 1887. The "heterotypic" chromosomes of his Figs. 22, 23 and 25 are accurately reproduced in several of our photographs of Plate IX. Since the ring chromosome was demonstrated by Flemming many investigators have identified them in a variety of forms. The ring chromosomes of our photographs of Plates VIII and IX are similar to those demonstrated by Henking,¹⁶ '91, in *Pyrrhocoris*; Moore, '95, in *Elasmobranchs*; Bolles Lee, '97, in *Helix*; von Klinckowström, '97, in *Prostheceraeus*, de Sinéty, '01, in *Orthoptera*, Schockaert, '04, in *Thysanozoon* and Montgomery, '04, in

¹⁶ Henking interprets the first division as a reducing division and the second as an equation division.

Plethodon. McClung, '00-'02, has shown rings, figures 8 and crosses in certain insects clearly demonstrated by excellent photographs. Helen Dean King, '01, has demonstrated the ring in the egg of *Bufo*, and her interpretation that the knob-like thickenings represent "the place of union of the two chromosomes that fused to form the ring" is confirmed by the photographs of our Plates VII and VIII; in *Allolobophora*, however, the rings do not divide longitudinally in the first division as in *Bufo*.

Many forms of the chromosomes in *Allolobophora* are similar to those demonstrated by Korschelt, '95, in *Ophryotrocha* and his interpretation of the first division separating two univalent chromosomes is confirmed by the photographs of our Plates VII, VIII and IX. Further details in which Korschelt's observations are supported by this egg are stated under the heading "Comparisons with other Annelids."

Archoplasm.—In earlier papers we have used Boveri's, '88, term archoplasm for that substance in the cytoplasm which in the youngest oöcytes is massed in different shapes close to the germinal vesicle (yolk nucleus of authors). As the egg grows this substance increases in amount, becomes distributed throughout the cytoplasm; after fertilization much of it gradually segregating to the periphery, and finally a large part of it contributing to the formation of the polar rings. We use the term archoplasm because at definite phases of the development of the egg the substance appears to contribute to the formation of asters and spindles and may thus be the homologue of Boveri's archoplasm. Progressive steps in the development of the substance from yolk nucleus to polar rings were illustrated by a series of photographs (Foot and Strobell, '01), but in the present paper we have reproduced only three sections of the earlier stages, merely to illustrate our interpretation. At the upper periphery of Photo. 12 about half of a very small oöcyte is shown, with a mass of archoplasm (yolk nucleus) at the opposite poles of its nucleus. The next stage represented in this paper is shown in Photo. 76, Plate IV, the archoplasm being somewhat removed from the nuclear membrane. A later stage is seen in Photo. 78, Plate IV, in which the substance is distributed throughout the cytoplasm, and Photo. 12 shows a like distribution in an older oöcyte. In recent investigations of this polar-ring substance we have attempted no analysis by complicated methods of staining, studying it only in the light of comparative fixation and aiming thus to demonstrate its presence in eggs in which, after some fixatives, its identity is questionable.

It is interesting to compare the effects of fixation on the two constituents of the cytoplasm, chromatic archoplasm and relatively achro-

matic cytoplasm, with the effects of fixation on the two *nuclear* constituents, chromatin and achromatic nucleoplasm, for in both cases the reaction to fixation is strikingly alike. Such a crude comparison is instructive only to illustrate the fact that both chromatin and archoplasm can be identified after some fixatives and obliterated after others, yet the specific character of chromatin is universally admitted, whereas the specific character of archoplasm has been very generally doubted. We do not mean to imply that the two substances, archoplasm and achromatic cytoplasm, are the sole constituents of the cytoplasm, any more than the chromatin and achromatic nucleoplasm may be the sole constituents of the nucleus. Archoplasm and achromatic cytoplasm are as much at the mercy of the fixatives as are the chromatin and achromatic nucleoplasm, and, like them, can be fused together into a network or into masses in which the constituents are indistinguishable, or they may be so separated that the two are readily differentiated. Such a differentiation is seen in the unstained sections of Photos. 68 and 69, Plate IV, the chromatic archoplasm and relatively achromatic cytoplasm being segregated into quite definite areas. We do not claim that such pronounced cases of segregations more nearly approximate the living condition, but they may be instructive as an aid to demonstrating the individuality and continuity of the substances during these stages, and the same may be said of some forms of segregated chromatin shown in many of our photographs of sections. The achromatic cytoplasm of Photos. 10, Plate I, 68 and 69, Plate IV, like the achromatic nucleoplasm, is a relatively homogeneous substance, but it becomes granular and chromatic when combined with the archoplasm (Photo. 13, Plate I, just as does the achromatic nucleoplasm when combined with chromatin (cf. the nucleoplasm of Photo. 34, Plate II, with that of the germinal vesicles of Plates II, III, IV). Aggregations of archoplasm like the three shown in Photo. 13 are distributed throughout the cytoplasm of the entire egg (cf. Foot and Strobell, '98, Photo. 9). Such aggregations are typical of chromo-acetic preparations and in the light of our recent study of the germinal vesicle we are convinced that the entire cytoplasm of such preparations represents an artificial combination of archoplasm and achromatic cytoplasm comparable to the fusing of the chromatin and achromatic nucleoplasm in the germinal vesicles of the same preparations (Photo. 34), and they support the observations of the investigators who question the specific nature of archoplasm. Such an interpretation would be supported also by Photo. 100, Plate VI, but a comparison of these two photographs (13 and 100) with 68 and 69, Plate IV, suggests that the individuality of the archoplasm, so clearly shown in the last two photographs, is only obscured

in Photos. 13 and 100. We believe the chromatic granular substance of the prophase (Photos. 68 and 69) is the same as the chromatic granular substance of the metaphase (Photo. 99, Plate VI). These eggs were killed in the same fixative, platino-osmic, and the substance can be recognized at the two stages. In Photo. 100 the substance has a very different distribution from that of Photo. 99, Plate VI, though the two eggs are at exactly the same stage of development, i. e., metaphase of the first spindle. In the chromo-acetic preparation (Photo. 100) the homogeneous achromatic cytoplasm is in the form of pronounced rays, combined in such a way with the archoplasm that the latter may be interpreted as cyto-microsomes. After some fixatives it certainly does assume the form of cyto-microsomes and in these cases its identification as a specific substance is possible only where it is accumulated into dense masses. Its interpretation as a specific substance or as an integral part of the cytoplasm depends upon its special manifestation after a given fixative and suggests that the opposing interpretations are largely a question of terms. In this egg we claim its individuality only on the ground that we think we can trace the substance with unbroken continuity from its earliest aggregation as yolk nucleus in the youngest oöcytes to the cleavage stages—a large part of it contributing to the formation of the polar rings. Aggregations of archoplasm not alone in chromo-acetic preparations, but in corrosive sublimate and many others are readily differentiated by double staining (Foot, '96), but this method obscures its presence when it is most evenly distributed throughout the egg, and for this reason study of comparative fixation has seemed the more profitable method to follow (Foot and Strobell, '00). When the oöcyte first order has reached its maximum growth it is especially difficult to differentiate the archoplasm. Its presence at this stage is demonstrated in Photos. 68 and 69, Plate IV, and Photo. 10, Plate I, shows an interesting segregation of the substance in the form of a "polar ring" which is not normally due until the pronuclear stage. This is a section of an oöcyte with the germlinal vesicle intact and the chromosomes not yet formed, a stage earlier than that shown in Photos. 68 and 69. There is a similar aggregation of archoplasm at the opposite pole of this egg and these two polar aggregations present a striking resemblance to many polar rings of the pronuclear stages which are not invariably in the form of a ring. This precocious polar segregation of the substance in Photo. 10 appears to us to demonstrate the presence of this definite substance in the egg during these early stages and the granular appearance of the archoplasm in this photograph is typical of all fixed material. The chromatic centers of asters fail to show this granular effect (Photos. 84,

86, 91, 92, Plate V, 99 to 110, Plate VI), but we do not think this necessarily means that these centers are devoid of archoplasm; it may indicate rather a definite chemical combination with the achromatic cytoplasm that causes a different morphological reaction to fixation (Foot, '96). An obvious contribution of archoplasm to the spheres is largely dependent on fixation, and in some cases it is aggregated into granular masses or heavy rays around the mark-zone (Foot and Strobell, '00), and again the mark-zone itself is granular and stains intensely.

Differentiation of a special chromatic substance in the cytoplasm of young oöcytes or spermatocytes is very common, but more rarely is this substance traced to the later stages of development. Among recent papers Voinov, '03, has traced a substance to the first spindle in *Cybister*, and finally to the Nebenkern. He has designated it as "zone interne" as distinguished from his "zone externe" (Figs. 29 and 30), and a comparison of his figures with our photographs leads to the impression that the archoplasm of *Allolobophora* is synonymous with his "*Mitochondria*" (Benda's) and "zone interne" combined (see his Figs. 35 and 39).

Data as to the specific nature of polar-ring substance have been presented by Wilson in his interesting paper on *Dentalium*, '04. He identifies an upper and lower polar area in the oöcyte and of these he says: "I believe it is probable that at least the lower protoplasmic area and probably also the upper disc are in a general way comparable to, if not identical with, the polar rings observed in the eggs of certain leeches and *Oligochaetes*." Of the lower polar area he says: "It is evident that material from the interior of the egg must flow into the lobe as it forms," and of the upper polar area he adds: "It is here again evident that an extensive flow of this material must take place from the interior of the egg" (pp. 12-15). These facts have a special bearing on our interpretation that the polar ring substance is distributed throughout the egg and later aggregates at the poles. (Foot and Strobell, '98.) Wilson interprets both areas as "specific cytoplasmic material."

In this connection Wheeler's work on *Myzostoma*, '97, is of special interest. He interprets certain phenomena at the upper pole of the egg as homologous to the polar rings of Annelids and he identifies in the oöcyte (Fig. 1) a denser area of protoplasm which strikingly resembles the yolk nucleus of *Allolobophora* and this he traces to the yolk-lobe (opposite pole), though he does not interpret the substance as yolk-nucleus, nor the yolk-lobe as homologous to a polar ring of Annelids. Conklin, however, in 1897, identifies at the vegetal pole of the egg of *Crepidula* a mass of hyaline substance which he homologizes to the yolk-lobe described by Mead in *Chælopterus* and to the polar rings of

Annelids.' He says: "I am convinced that this peculiar body is homologous with the problematical lobe which is described by Mead, '95, in the egg of *Chatopterus* and further it is probably identical with the polar rings observed by Whitman, '78, in *Clepsine* and since then by various authors in different Annelids."

COMPARISONS WITH OTHER ANNELIDS.

In the oogenesis of Annelids there has been very little work done on the prophases of the first maturation spindle.

Among the *Lumbricidae* we have not found any record of observations on these stages, but in the spermatogenesis of *Lumbricus terrestris* Calkins, '95, has studied the prophases of the first division and the two species, *Allolobophora* and *Lumbricus*, are in accord in showing a spireme with a longitudinal furrow. In *Lumbricus*, however, the spireme divides transversely into the full number of somatic chromosomes, and there is, therefore, no numerical reduction of the chromosomes by two univalent chromosomes remaining attached end to end as in *Allolobophora*.

In the first spindle of *Lumbricus* there are sixteen tetrads, the spermatocytes second order receiving each sixteen double chromosomes. Calkins adds: "Whether this is a reducing division in Weismann's sense cannot be ascertained."

Vejdovsky's and Mrázek's recent valuable work, '03, on *Rhynchelmis* is confined to later stages, the material being unfavorable for the study of the prophases of the first maturation spindle. Of these stages they say: "Das Studium der ersten Vorgänge der Eireifung ist schon technisch sehr zeitraubend, wenn man auf den endlosen Schnittserien durch die einige Zentimeter langen von Eiern prall angefüllten vorderen Abschnitte des Wurmleibes stets nur entweder noch ruhenden Kernen oder den bereits fertigen Reifungsspindeln begegnet" (pp. 454 and 455). They have, however, supplemented their work on *Rhynchelmis* by a study of the prophases of the first spindle in *Tubifex*, *Limnodrilus* and *Ilyodrilus*, but their results are demonstrated by a single text figure showing a germinal vesicle with tetrads. In explanation of this figure they say: "Den ganzen Vorgang der Chromosomenbildung könnten wir nicht verfolgen. Erst in späteren Stadien fanden wir die in Textfigur 3 abgebildeten Formen der Chromosomen. Es sind dies Gebilde, die wie aus zwei dicht an einander gelegten sickel- oder biskuitförmigen Teilen zusammengesetzt erscheinen. Ein Vergleich mit den an anderen Objekten gewonnenen Resultaten führt zu dem Ergebnisse, das wir hier längsgespaltene doppelwertige Elemente vor uns haben, die den typischen

Vierergruppen entsprechen. Wie die Abbildung zeigt, kann die Form der einzelnen Gruppen etwas variieren, doch muss ausdrücklich bemerkt werden, dass wir Ringbildung niemals beobachten könnten. Dagegen sind kreuz- oder x-förmige Figuren die häufigsten."

In Text Figs. 4 and 7 they show 37 different forms of the chromosomes of the first maturation spindle of *Rhynchelmis*. In our Photo. 72, Plate IV, there is an exact duplicate of one of the chromosomes in their Text Fig. 4, and a comparison of their other figures with our photographs of the first spindle of *Allolobophora* (Plate IX and our Text Fig. 4 of an earlier paper, '98), show a suggestive similarity in form. Vejdovsky and Mrázek state, and their Text Fig. 5 demonstrates, that only the central part of the first maturation spindle of *Ilyodrilus* is of nuclear origin, but in *Allolobophora* a much larger proportion of the spindle is derived from the achromatic nucleoplasm of the germinal vesicle (see Photos. 84 to 89, Plate V). On this point *Allolobophora* is more in accord with Gathy's, '00, observations on *Tubifex*, though the membrane of the germinal vesicle persists longer in *Tubifex* than in *Allolobophora*. Gathy's Fig. 11 shows the first spindle nearly at the metaphase and yet the membrane of the germinal vesicle is almost intact, whereas in *Allolobophora* the nuclear membrane entirely disappears before the spindle reaches the metaphase. Our Photos. 84 to 89, Plate V, show part of the membrane of the germinal vesicle persisting until both centrioles and asters are present at opposite poles, though not developed to the stage shown in Gathy's Figs. 10 or 11; these photographs indicate, however, that the achromatic nucleoplasm of the germinal vesicle of *Allolobophora*, like *Tubifex*, contributes to a large part of the first maturation spindle. *Allolobophora* further supports Gathy's observations as to the independent origin of the two centrioles, their first appearance close to the nuclear membrane and the indication that the spindle is formed under their influence. Gathy omits other important details in the formation of the spindle and his Figs. 8, 9 and 10 demonstrate that he has not observed the successive steps of the development of the chromatin of the germinal vesicle into the chromosomes.

Among the Annelids these stages have been most thoroughly investigated by Korschelt in *Ophryotrocha*, '95, and *Allolobophora* corroborates almost every detail of the process Korschelt describes. In both *Oligochaetes* the chromatin forms a skein, though in *Ophryotrocha* the longitudinal furrow does not appear until after the chromosomes are formed. The skein divides transversely into chromosomes, in *Ophryotrocha* these being univalent, whereas in *Allolobophora* they are bivalent, as a rule remaining bivalent until separated at the anaphase of the first spindle.

In both *Oligochates* the chromosomes have a distinct longitudinal furrow, which has persisted in *Allolobophora* from the skein stage, and in both forms the first division separates two univalent chromosomes. In these two Annelids the achromatic nucleoplasm contributes to the formation of the spindle fibers, the fibers forming within the germinal vesicle while its membrane is partly intact, and the centriole in both cases is first seen outside the germinal vesicle close to its membrane, though in *Ophryotrocha* the two arise by division of one, while in *Allolobophora* they are first seen at opposite poles of the vesicle.

Thalassema.—Griffin's interesting paper on the maturation and fertilization of the egg of *Thalassema*, '99, gives a clear demonstration of the prophases of the first maturation spindle. These are reproduced in his Figs. 1 to 12, but Griffin demonstrates no spireme in the germinal vesicle, and he neither figures nor describes stages answering to the stages shown in our Photos. 111 to 115, Plate VII, and in Korschelt's, '95, Figs. 67 to 74. He figures a spireme only in the nuclei of "minute ova," the size of these nuclei in relation to the germinal vesicles of later stages showing them to be the young nuclei emerging from the telophase of the last oögonial division, and the spireme of these nuclei is not comparable to the spireme demonstrated in our photographs of Plate VII. They can be compared only to similar minute cells in the ovary of *Allolobophora*, stages with which we are not concerned in the present paper. In the text Griffin describes the spireme of the nuclei of these minute cells as showing an occasional longitudinal split and dividing transversely into bivalent chromosomes at the *beginning of the growth period* (Fig. 2), these chromosomes persisting "as double rods *throughout the entire growth period*" (p. 605). In *Allolobophora* the chromosomes do not persist through the growth period nor can any indication of the aggregation of the diffused chromatin into the spireme of Photos. 111 to 115 be demonstrated until some time after the germinal vesicle has attained its maximum size (see p. 217 for details). The two Annelids agree, however, as to the form of the final tetrads. Griffin's Text Figs. 1 and 2 show chromosomes in the form of rings, figures 8, crosses, etc., which are strikingly like those in many of our photographs, though their origin is apparently very different. In *Thalassema* the spireme divides transversely into half the number of somatic segments, these bivalent chromosomes differing, however, from *Allolobophora* in the important point that their bivalent character is expressed by a longitudinal division of each, instead of two univalent chromosomes being attached end to end, as in *Allolobophora*. Thus the rings, figures 8, etc., which are common for the two Annelids have a different origin, necessitating a different

interpretation for the first division, in *Thalassema* the first division being longitudinal (giving to each cell *one-half of every univalent chromosome*), and in *Allolobophora* transverse (giving to each cell *entire univalent chromosomes*, each receiving one-half the somatic number). Griffin has clearly stated his results in the following summary:

"1. By longitudinal fission and transverse segmentation of the spireme thread, there arise 12 (reduced number) ellipse-shaped chromatin masses.

"2. These persist throughout the growth period of the egg.

"3. During prophase they concentrate into crosses, the arms of which are tight loops.

"4. In the first polar division these are drawn out again into ellipses which divide to form daughter-V's (equation division).

"5. The V's break apart at the angle in the second polar division (reducing division)," p. 612.

The persistence of the chromosomes throughout the entire growth period, during the time that the nuclear reticulum is gradually developing, led Griffin to the conclusion that "its development is independent of the chromosomes which are passive during its growth" (p. 604), and Griffin's conclusions as to the independence of these two substances are supported by our observations on *Allolobophora*. The two Annelids are further in accord as to the first appearance of the maturation centrioles. In both types they are first seen as minute asters close to the germinal vesicle, though in *Thalassema* they are closer to each other than we have yet found them in *Allolobophora*. This independence of the centrioles accords with Mead's observation on *Chætopterus*, '98 (cf. our Photos. 81 and 82, Plate V, with Mead's Figs. 8 and 9). The two extremely small centrioles of the above-mentioned photographs show a more marked independence of origin than those figured by Mead, for they are at opposite poles of the germinal vesicle, while closer to its membrane and at an earlier stage of development than those figured for *Chætopterus*. These primary asters of *Chætopterus* "arise at some distance from the wall of the germinal vesicle," and Mead adds: "I am not prepared to say at present whether the primary asters are formed by the further growth and specialization of two of the secondary asters or by the union and coalescence of several." These secondary multiple asters which Mead has demonstrated in his Fig. 7, he has shown to be normal in *Chætopterus*, having watched the phenomena in living eggs, which continued to develop after the multiple asters had disappeared. In *Allolobophora* we have found only one egg showing structures that could be interpreted as multiple asters, but the egg was unquestionably pathological, the germinal

vesicle had broken down and its contents scattered throughout the cytoplasm. These structures in *Allolobophora* resemble some of the asters in *Cerebratulus* which Kostanecki interprets as expressions of a pathological condition. They show, however, no evidence that they have originated by division of the normal aster as Kostanecki, '02, interprets those of *Cerebratulus*, they indicate rather that their irregular dense centers are small aggregations of dispersed nuclear substance, around which cytoplasmic rays focus.

Mead gives no account of the development of the nine chromosomes which he finds in the first maturation spindle. These stages are omitted also in Wheeler's work on *Myzostoma*, '97, where he first figures the chromosomes as 12 tetrads suspended in the chromatic network of the germinal vesicle. These are composed of two rods swollen at their ends, and of these Wheeler says: "I have not studied the origin of the double rods in the germinal vesicle so that I am unfortunately unable to pass an opinion on the nature of the division in the first spindle. In the case of the second spindle, however, I feel confident that there is a longitudinal splitting of each of the single chromatin rods remaining in the egg after the formation of the first polar body" (p. 51). *Allolobophora* supports Wheeler's observations as to the occasional persistence of part of the membrane of the germinal vesicle until the spindle is formed (cf. Photos. 84 to 87, Plate V, with Wheeler's Fig. 63). In *Myzostoma* the two centrioles are first seen near the membrane of the germinal vesicle and in close relation to each other, being "connected by a delicate achromatic bridge." Wheeler's Figs. 3 to 5 demonstrate the gradual separation of these centrioles to form the poles of the first spindle.

DECEMBER 1, 1904.

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EXPLANATION OF PLATES.

All the photographs are of the egg of *Allolobophora fœtida* and were taken by the method described in Zeit. f. wiss. mik., Bd. XVIII, 1901, "A new method of focusing in photo-micrography," Foot and Strobell.

The Zeiss apo. 2 mm. immers. lens 140 apr. and compensating ocular 4 were used, and for the one thousand magnification a camera draw of 25 $\frac{3}{4}$ inches.

Many of the photographs were taken on the Seed No. 27 and the Agfa Isolar plates. With these rapid plates an exposure of only one to three minutes was required even on cloudy days. In the photographs of Plates VII, VIII and IX the wider area of accurate focus demanded by the subjects made it necessary to close the substage diaphragm to a point requiring doubling the time of exposure.

Only a small part of each section is reproduced, except in photos. 68 and 69.

The reproductions of Plates I, IV and VI are by the half-tone process, and Plates II, III, V, VII, VIII and IX are by the Rotograph process.

PLATE I.

The oöcytes shown in Photos. 1 to 11 inclusive were fixed in platino-osmic (Hermann's fluid without acetic acid) and stained with iron hæmatoxylin followed by dilute Bismarck brown. All the sections are 2 $\frac{1}{2}$ μ .

PHOTO. 1. $\times 1000$. First of four consecutive sections of a germinal vesicle of an oöcyte first order from a receptaculum ovorum, showing chromatic filaments stained black, and faintly stained nucleoplasm.

PHOTO. 2. $\times 1000$. Next section to above.

PHOTO. 3. $\times 1000$. Next section to Photo. 2 showing an *accessory nucleolus* stained black like the chromatic filaments. This is the only *accessory nucleolus* in this germinal vesicle.

PHOTO. 4. $\times 1000$. Next section to above showing the *principal nucleolus* which stained yellow by the Bismarck brown. This nucleolus is also present in the two following sections.

PHOTO. 5. $\times 1000$. Section of a germinal vesicle of an oöcyte first order from the receptaculum ovorum, showing black chromatic filaments, and large yellow nucleolus with small black granules (these granules are lost in some of the reproductions). This section was first photographed unstained and the granules in the nucleolus were as black and sharply differentiated as in the stained preparation.

PHOTO. 6. $\times 1000$. First of four serial sections of a germinal vesicle of an oöcyte first order from receptaculum ovorum showing black chromatic filaments and one *accessory nucleolus*, also stained black.

PHOTO. 7. $\times 1000$. Second section from above showing the *principal nucleolus*. This nucleolus stained brownish yellow and is present in the two following sections.

PHOTO. 8. $\times 1000$. Next section to Photo. 7 showing the same large nucleolus and a second *accessory nucleolus* stained black, at the upper left hand periphery of the germinal vesicle.

PHOTO. 9. $\times 1000$. Second section from Photo. 8 showing a third *accessory nucleolus* which in this section is attached to one of the chromatic filaments.

PHOTO. 10. $\times 1000$. Section of an oöcyte first order from a receptaculum ovorum, at the same stage of development as those shown in Photos. 1 to 9. The archoplasmic granules are aggregated at two poles of the oöcyte bearing a striking resemblance to the polar rings of the ripe egg. Only one pole is shown in the photograph.

PHOTO. 11. $\times 1000$. Section of germinal vesicle of an oöcyte first order from a receptaculum ovorum, showing black chromatic filaments and one *accessory nucleolus* attached to the longest filament. This was the only *accessory nucleolus* in this germinal vesicle, and the *principal nucleolus* stained brownish yellow as in the other oöcytes.

PHOTO. 12. $\times 710$. Young oöcyte from ovary showing the large vacuolated nucleolus and well defined areas of archoplasm in the cytoplasm. On the periphery of the photograph is shown part of a very small oöcyte with aggregations of archoplasm (yolk nucleus) at opposite poles of the germinal vesicle. Fixative, corrosive sublimate (saturate). Stain, iron haematoxylin.

PHOTO. 13. $\times 1000$. Part of periphery of oöcyte first order from a receptaculum ovorum. The two membranes, with an indication of the substance between, are distinctly shown. The archoplasm is fused with the cytoplasm in a form characteristic of chromo-acetic fixation, c. f. Photo. 10 for platinosmic fixation. Fixative, chromo-acetic. Stain, iron haematoxylin followed by dilute Bismarck brown.

PHOTO. 14a. $\times 1000$. Nucleolus with black granules in large *unstained* oöcyte from ovary. The granules resemble the black granules found in the cytoplasm of the same preparation. Fixative, chromo-acetic followed by osmic acid, which blackened the granules of the nucleolus and cytoplasm.

PHOTO. 14b. $\times 1000$. Nucleolus in a large *unstained* oöcyte from distal end of ovary. Fixative, Hermann's fluid.

PHOTOS. 15, 16 and 17. $\times 700$. Vacuolated nucleoli from large *unstained* oöcytes from ovary. Fixative, Hermann's fluid.

PHOTO. 18. $\times 700$. Vacuolated nucleolus in medium sized *unstained* oöcyte from ovary. Fixative, Hermann's fluid.

PHOTOS. 19, 20 and 21. $\times 700$. Vacuolated nucleoli in large *unstained* oöcytes from ovary. Photo. 21 shows both a *principal* and *accessory nucleolus*. Fixative, picro-nitric followed by osmic acid.

PLATE II.

The section of Photo. 26 is $2\frac{1}{2} \mu$. All the others are $2\frac{1}{2} \mu$.

PHOTOS. 22-25. $\times 1000$. Four sections selected from fourteen of a germinal vesicle of an oöcyte first order from a receptaculum ovorum. The *principal nucleolus* is present in three consecutive sections, two of them being shown in Photos. 22 and 23. Photo. 23 shows one *accessory nucleolus* attached to the largest chromatic filament. Fixative, Rabl's picro-sublimate. Stain, iron haematoxylin, followed by dilute Bismarck brown.

PHOTO. 26. \times about 1100. Section of upper pole of a first maturation spindle with sphere and centriole in fertilized oöcyte from freshly deposited cocoon. There is a centriole at the lower pole of this spindle, but we selected this photograph as more interesting for reproduction, because it is much more difficult to demonstrate a centriole at the upper pole, when it is close to the periphery. Fixative, Flemming's fluid without acetic acid. Stain, iron hæmatoxylin, followed by dilute Bismarck brown.

PHOTOS. 27 and 28. \times 1000. Two sections of the same germinal vesicle of an oöcyte first order from a receptaculum ovorum. There are four intervening sections between the two reproduced here and the *principal nucleolus* of Photo. 27 shows no connection with the chromatic filaments. Fixative, platino-osmic. Stain, iron hæmatoxylin followed by dilute Bismarck brown.

PHOTO. 29. \times 1000. Section of a germinal vesicle of an oöcyte first order from a receptaculum ovorum, showing the large vacuolated nucleolus and the smaller *accessory nucleolus*. Fixative, Picro-sublimate. Stain, iron hæmatoxylin, followed by dilute Bismarck brown.

PHOTO. 30. \times 1000. Section of a germinal vesicle of an oöcyte first order from a receptaculum ovorum showing chromatic filaments in transverse section and two short granular filaments crossing each other. The large vacuolated nucleolus is in the 3rd, 4th and 5th sections from that of Photo. 30. Fixative, corrosive-acetic (5 per cent acetic). Stain, iron hæmatoxylin followed by dilute Bismarck brown.

PHOTO. 31. \times 1000. Section of a germinal vesicle of an oöcyte first order from a receptaculum ovorum showing the large vacuolated nucleolus and an early stage of the segregation of the chromatin into chromatic filaments. Fixative, corrosive sublimate. Stain, iron hæmatoxylin.

PHOTO. 32. \times 1000. Section of lower pole of second maturation spindle showing four of the eleven chromosomes approaching the lower pole. This section was photographed to show a precocious formation of one of the eleven vesicles not due normally until the telophase. The centriole is in the next section. Fixative, chromo-acetic. Stain, iron hæmatoxylin.

PHOTO. 33. \times 1000. Section of first maturation spindle, showing five of the eleven chromosomes. This egg is one of eight found in an immature cocoon encircling the clitellum of a worm. The four chromosomes which are on the same plane are clearly defined and show a decided difference in size. We have six photographs of this spindle showing all the eleven chromosomes but cannot spare space for more than one reproduction. Fixative, corrosive acetic (10 per cent acetic.) Stain, iron hæmatoxylin, followed by dilute Bismarck brown.

PHOTO. 34. \times 1000. Section of a germinal vesicle of an oöcyte first order from a receptaculum ovorum, showing the typically injurious effect of chromo-acetic on the nucleoplasm. This photograph shows a distinct net knot resembling a large nucleolus with two vacuoles, but it is unmistakably an artefact, for the *principal nucleolus* of this egg is present in the seventh section from the one reproduced in this photograph. Stain, iron hæmatoxylin followed by dilute Bismarck brown.

PHOTO. 35. \times 710. Section of a vacuolated nucleolus of an oöcyte from the ovary. Fixative, corrosive sublimate followed by osmic acid. Unstained.

PHOTO. 36. $\times 710$. Section of a ring nucleolus of an oöcyte from the ovary. Fixative, Lindsay Johnson's fluid. Stain, acid fuchsin.

PHOTO. 37. $\times 710$. Section of a vacuolated nucleolus. A small *accessory nucleolus* is also present. Fixative and stain same as Photo. 36.

PHOTO. 38. $\times 710$. Section of a ring shaped nucleolus of an oöcyte from the ovary. Fixative, Merkel's fluid followed by osmic acid. Stain, iron hæmatoxylin.

PHOTO. 39. $\times 710$. Section of a vacuolated nucleolus from an ovarian oöcyte and near it an *accessory nucleolus*. Fixative, picro-sulphuric followed by osmic acid. Unstained.

PHOTO. 40. $\times 710$. Section of a vacuolated nucleolus from an ovarian oöcyte and near it a small *accessory nucleolus*. Fixative, picro-sulphuric followed by osmic acid. Unstained.

PHOTO. 41. $\times 710$. Section of a vacuolated nucleolus from an ovarian oöcyte. This nucleolus appears to be surrounded by a definite membrane and this is shown in three consecutive sections. Fixative, picro-sulphuric. Stain, iron hæmatoxylin.

PHOTO. 42. $\times 710$. Section of a vacuolated nucleolus from medium sized ovarian oöcyte. Fixative, corrosive sublimate. Stain, iron hæmatoxylin.

PHOTO. 43. $\times 710$. Section of a ring shaped nucleolus in oöcyte from a receptaculum ovorum. Fixative, corrosive acetic (10 per cent acetic). Stain, iron hæmatoxylin followed by dilute Bismarck brown.

PHOTO. 44. $\times 710$. Section of a ring shaped nucleolus in an ovarian oöcyte. Fixative, Graf's picro-formalin followed by osmic acid. Stain, iron hæmatoxylin.

PHOTO. 45. $\times 710$. Section of a crescent shaped nucleolus in ovarian oöcyte. Fixative, Graf's picro-formalin followed by osmic acid. Unstained.

PLATE III.

All sections $2\frac{1}{2} \mu$.

PHOTOS. 46 to 49. $\times 1000$. Four sections selected from eighteen of a germinal vesicle of an oöcyte first order from a receptaculum ovorum. Photos. 46 to 48 are consecutive sections, and Photo. 49 is the third section beyond Photo. 48 and is one of three consecutive sections in which the large vacuolated nucleolus is present. There are two *accessory nucleoli* also present in this section. All these sections show the chromatin segregated into more or less definite filaments and Photo. 47 shows a filament with what appears to be a longitudinal split. Fixative, saturate corrosive sublimate. Stain, iron hæmatoxylin.

PHOTO. 50. $\times 1000$. Peripheral section of a germinal vesicle of an oöcyte first order from a receptaculum ovorum showing a ring shaped nucleolus. Fixative, platino-osmic. Stain, iron hæmatoxylin followed by dilute Bismarck brown.

PHOTOS. 51 and 52. $\times 1000$. Two consecutive sections of a germinal vesicle of an oöcyte first order from a receptaculum ovorum. Each section shows a ring chromosome, the two differing greatly in size. The *principal nucleolus* is present in this germinal vesicle and intact. Fixative, saturate corrosive sublimate. Stain, Bismarck brown.

PHOTOS. 53 and 54. $\times 1000$. Each of these photographs shows one of three consecutive sections of a vacuolated nucleolus in an oöcyte first order from a receptaculum ovorum. Fixative, saturate corrosive sublimate. Stain, iron haematoxylin.

PHOTO. 55. $\times 710$. Section of a nucleolus in an ovarian oöcyte. Fixative, bichromate-cupric sulphate. Stain, iron haematoxylin.

PHOTO. 56. $\times 1000$. Part of a section of a germinal vesicle of an oöcyte first order from a receptaculum ovorum showing the *principal* and the *accessory nucleoli*. Fixative, platino-osmic. Stain, iron haematoxylin followed by dilute Bismarck brown.

PHOTO. 57. $\times 710$. Section of a vacuolated nucleolus in an ovarian oöcyte. Fixative, bichromate-cupric sulphate. Stain, iron haematoxylin.

PHOTO. 58. $\times 1000$. Section of a germinal vesicle of an oöcyte first order from a receptaculum ovorum. This is one of four consecutive sections of the *principal nucleolus*. Fixative, corrosive acetic (5 per cent acetic). Stain, iron haematoxylin followed by Bismarck brown.

PHOTOS. 59 to 62. $\times 1000$. Four sections selected from sixteen of a germinal vesicle of an oöcyte first order from a receptaculum ovorum. The first three sections are consecutive. The *principal nucleolus* of Photos. 59 and 60 shows no connection with the chromatic filaments. Fixative, Rabl's picro-sublimate. Stain, iron haematoxylin followed by Bismarck brown.

PHOTOS. 63 to 65. $\times 1000$. Three sections of a germinal vesicle of an oöcyte first order from a receptaculum ovorum. Photo. 63 shows an *accessory nucleolus* with transverse sections of a chromosome at opposite sides of it. The *principal nucleolus* is in the sixth section from the *accessory nucleolus*. In Photo. 64 there is a chromosome in the form of a figure eight and in Photo. 65 a rod shaped chromosome and transverse sections of two more. Fixative, saturate corrosive sublimate. Stain, iron haematoxylin.

PHOTO. 66. $\times 1000$. Section of a germinal vesicle of an oöcyte first order from a receptaculum ovorum showing chromatic filaments and an *accessory nucleolus* attached to a chromatic filament. Fixative, saturate corrosive sublimate. Stain, iron haematoxylin.

PHOTO. 67. $\times 710$. Section of a nucleolus of an oöcyte from the ovary. Fixative, bichromate-cupric sulphate. Stain, iron haematoxylin.

PLATE IV.

The section of Photo. 76 is 3μ , of Photo. 77 2μ , and all others on this plate are $2\frac{1}{2} \mu$.

PHOTOS. 68 to 73. $\times 1000$. Six consecutive sections of an oöcyte first order, from the receptaculum ovorum. Photos. 68 to 69 are entire sections unstained. They demonstrate very clearly the presence of the polar-ring substance (yolk-nucleus, archoplasm) and the deuto-plasmic granules blackened by the osmic in the fixative (platino-osmic). In Photos. 70 to 73 only the germinal vesicle and a small part of the surrounding cytoplasm are reproduced. Photo. 70 shows a group of chromosomes, Photo. 71 the *principal nucleolus* and a small *accessory nucleolus*, Photo. 72 a large *accessory nucleolus* and a cross shaped chromosome, and Photo. 73 a second section of the *accessory nucleolus* of Photo. 72. Photos. 70 to 73 are stained with iron haematoxylin followed by dilute Bismarck brown.

PHOTO. 74. $\times 710$. Section of a vacuolated nucleolus of an oöcyte from the ovary. Fixative, corrosive-acetic (20 per cent acetic) followed by osmic acid. Unstained.

PHOTO. 75. $\times 710$. Section of a nucleolus with dark granules. From an oöcyte in the ovary. Fixative, picro-acetic followed by osmic acid. Unstained.

PHOTO. 76. $\times 710$. Section of a small oöcyte in the ovary showing the nucleolus and yolk nucleus. Fixative, saturate corrosive sublimate followed by osmic acid. Unstained.

PHOTO. 77. \times about 1100. Section of a nucleolus of an oöcyte from the ovary. Fixative, Flemming's fluid (strong). Stain, iron hæmatoxylin followed by dilute Bismarck brown.

PHOTO. 78. $\times 710$. Section of a young oöcyte in the ovary showing a ring nucleolus and archoplasm (yolk-nucleus) in the cytoplasm. Fixative, saturate corrosive sublimate. Stain, iron hæmatoxylin.

PHOTO. 79. $\times 1000$. Section of a ring nucleolus of an oöcyte from the ovary. Fixative, Merkel's fluid followed by osmic acid. Stain, iron hæmatoxylin. (Photo. 80 was omitted by mistake.)

PLATE V.

All sections $2\frac{1}{2} \mu$

PHOTOS. 81 and 82. $\times 1000$. Two sections of a germinal vesicle of an oöcyte first order from the receptaculum ovorum. This vesicle was cut into nineteen sections and the two reproduced here were fifteen sections apart and both very close to the periphery of the germinal vesicle; the next section on each side being the last to show the germinal vesicle. These photographs show the earliest appearance of the centrioles and asters at opposite poles of the germinal vesicle. The centriole in Photo. 81 is still in contact with the membrane of the vesicle and the centriole in 82 is very close to the membrane. Fixative, chromo-acetic. Stain, iron hæmatoxylin followed by dilute Bismarck brown.

PHOTO. 83. $\times 1000$. Section of a germinal vesicle of an oöcyte first order from the receptaculum ovorum. The centriole and aster are further developed than those in Photos. 81 and 82 and not so close to the membrane of the germinal vesicle. The other centriole is eleven sections from the one in this photograph. Fixative, Boveri's picro-acetic. Stain, iron hæmatoxylin followed by dilute Bismarck brown.

PHOTOS. 84 to 89. $\times 1000$. Consecutive sections of a germinal vesicle of an oöcyte first order from a receptaculum ovorum. 86 and 87 are photographs of the same section, 86 being taken on the plane of the small centriole, and 87 on a lower plane to show the rest of the chromosomes. The two centrioles are seen at opposite poles in Photos. 84 and 86. There are two *accessory nucleoli* in Photo. 89. Traces of the persisting membrane of the germinal vesicle are seen in nearly all the sections. Fixative, Rabl's picro-sublimate. Stain, iron hæmatoxylin followed by dilute Bismarck brown.

PHOTOS. 90 and 91. $\times 1000$. Two planes of the same section of a first maturation spindle in an oöcyte from a receptaculum ovorum, showing four of the eleven chromosomes. Both centrioles are in this section but not on the same plane as the two chromosomes of Photo. 90. One centriole is shown in Photo. 91, which was taken before staining and focused for the unstained

centriole at the upper pole, the centriole of the lower pole was not on the same plane. Photo. 91 shows two unstained chromosomes on a different plane from the two in Photo. 90. Fixative, platino-osmic. Stain, Photo. 90 iron haematoxylin followed by dilute Bismarck brown. Photo. 91, unstained.

PHOTOS. 92a and b. $\times 1000$. Two consecutive sections of a first maturation spindle of a fertilized oöcyte in a freshly deposited cocoon. We have twelve photographs of this spindle showing all the eleven chromosomes and both centrioles. In the two selected for reproduction both centrioles are shown and a few of the chromosomes. 92a was focused for the centriole, sacrificing a sharp definition of the chromosome, and 92b was focused for the peripheral centriole, only one of the four chromosomes being on the same plane. Fixative, chromo-acetic. Stain, iron haematoxylin.

PHOTOS. 93 to 97. $\times 710$. Sections of nucleoli of oöcytes from ovary. Fixative, Rabl's picro-sublimate. Stain, iron haematoxylin.

PHOTOS. 98a and b. $\times 710$ and 1000. Section of a nucleolus of an oöcyte from the ovary. Fixative, Platino-osmic. 98a unstained. 98b stained with iron haematoxylin.

PLATE VI.

The sections in Photos. 99 and 105 are $3\ \mu$, those of 102 and 106 are $5\ \mu$, all the others are $2\frac{1}{2}\ \mu$.

PHOTO. 99. $\times 710$. Unstained section of an unfertilized oöcyte from a freshly deposited cocoon showing the first maturation spindle at the metaphase, with two of the eleven chromosomes. The peripheral centriole is not visible in this unstained section, but the centriole at the lower pole of the spindle could be clearly seen. Fixative, platino-osmic.

PHOTO. 100. $\times 1000$. Section of an unfertilized oöcyte from an immature cocoon still encircling the clitellum of the worm. This photograph shows the peripheral pole of the first maturation spindle with the centriole and thread-like rays typical of chromo-acetic preparations. The two membranes of the egg with the substance between are clearly defined, and this is shown also in Photo. 99. Fixative, chromo-acetic. Stain, iron haematoxylin followed by dilute Bismarck brown.

PHOTO. 101. $\times 1000$. Section showing the centriole at lower pole of a first maturation spindle of an oöcyte from the receptaculum ovorum. Fixative, Boveri's picro-acetic. Stain, iron haematoxylin followed by dilute Bismarck brown.

PHOTO. 102. $\times 710$. Section showing the centriole at peripheral pole of a first maturation spindle of an oöcyte from the receptaculum ovorum. Fixative, chromo-acetic. Stain, iron haematoxylin.

PHOTO. 103. $\times 1000$. Section showing the centriole at the lower pole of a second maturation spindle of a fertilized oöcyte from a cocoon. Fixative, chromo-acetic. Stain, iron haematoxylin.

PHOTO. 104. $\times 1000$. Section of the second maturation spindle of a fertilized oöcyte from a cocoon, showing the centriole at the lower pole, but not in the centre of the sphere. Fixative, chromo-acetic. Stain, iron haematoxylin.

PHOTO. 105. \times about 1000. Section showing the centriole at the lower pole of a second maturation spindle of a fertilized oöcyte from a cocoon. Fixative, saturate corrosive sublimate. Stain, iron haematoxylin.

PHOTO. 106. $\times 1000$. Section showing the centriole at the peripheral pole of a first maturation spindle, of an oöcyte from the receptaculum ovorum. Fixative corrosive acetic (10 per cent). Stain, iron hæmatoxylin followed by dilute Bismarck brown.

PHOTO. 107. $\times 1000$. Section of the spindle of one of two small cells of a three celled egg from the cocoon, showing one of the two centrioles. Fixative, chromo-acetic. Stain, iron hæmatoxylin.

PHOTO. 108. $\times 710$. Section showing the centriole at lower pole of the second maturation spindle of a fertilized oöcyte from a cocoon. Fixative, Merkel's fluid. Stain, iron hæmatoxylin.

PHOTO. 109. \times about 1100. Section showing centriole at the lower pole of a second maturation spindle of a fertilized oöcyte from a cocoon. Fixative chromo-acetic. Stain, iron hæmatoxylin.

PHOTO. 110. $\times 710$. Section of a second cleavage spindle of an egg from a cocoon. Both centrioles are shown though they are not on exactly the same plane. Fixative, chromo-acetic followed by osmic acid. Stain, iron hæmatoxylin.

PLATES VII, VIII and IX.

The preparations shown in the photographs of these plates are from oöcytes from the receptaculum ovorum (except Photo. 128), and were obtained by the method described on p. 200.

The preparations were stained with Bismarck brown.

Magnification of all the photographs, 1000 diameters.

PHOTOS. 111 and 113. Two germinal vesicles each showing a *principal nucleolus*, one *accessory nucleolus* and a fine chromatin thread.

PHOTO. 112. Germinal vesicle showing a pathological aggregation of the chromatin.

PHOTOS. 114 and 115. Two germinal vesicles showing each a *principal nucleolus* and a longitudinally split spireme. In Photo. 114 there is one, and in Photo. 115 there are two *accessory nucleoli*.

PHOTOS. 116 to 124. Germinal vesicles showing different forms of the eleven bivalent chromosomes immediately after the transverse division of the spireme, in Photo. 116, two of the bivalent chromosomes are still attached end to end. Nearly all these preparations show a longitudinal split in some of the chromosomes, and in Photos. 116, 118, 121, 122, 123 and 124 both the *principal* and the *accessory nucleoli* are present.

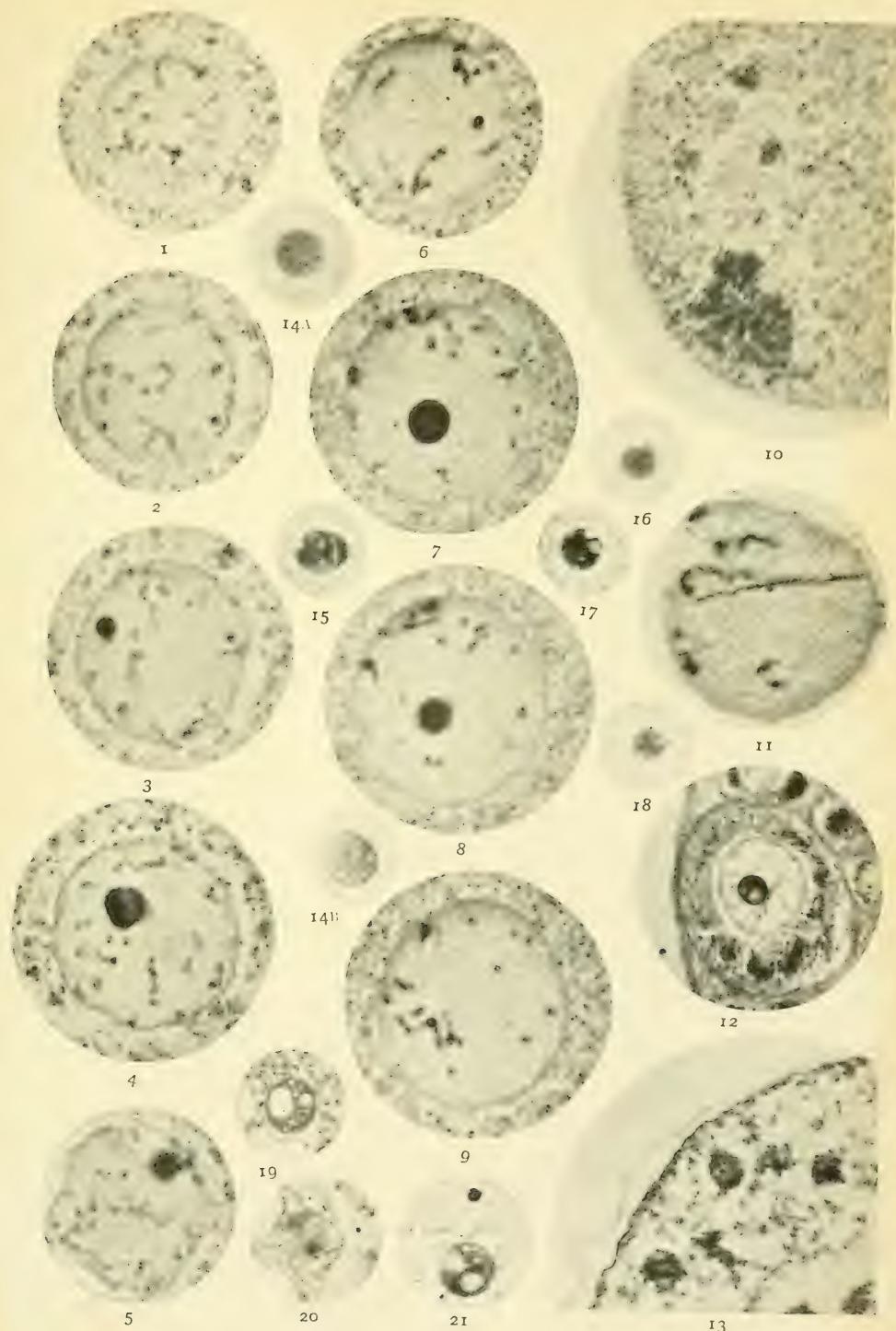
PHOTOS. 125, 126, 127, 129 and 130 show the eleven bivalent chromosomes arranged with more or less regularity in the equator of the first maturation spindle. For detailed description of these photographs, see p. 217.

PHOTO. 128. The eleven chromosomes nearly in the equator of the first maturation spindle of an oöcyte from an immature cocoon.

PROPHASES OF FIRST MATURATION SPINDLE OF
ALLOLOBOPHORA FOETIDA.

FOOT & STROBELL PHOTOS.

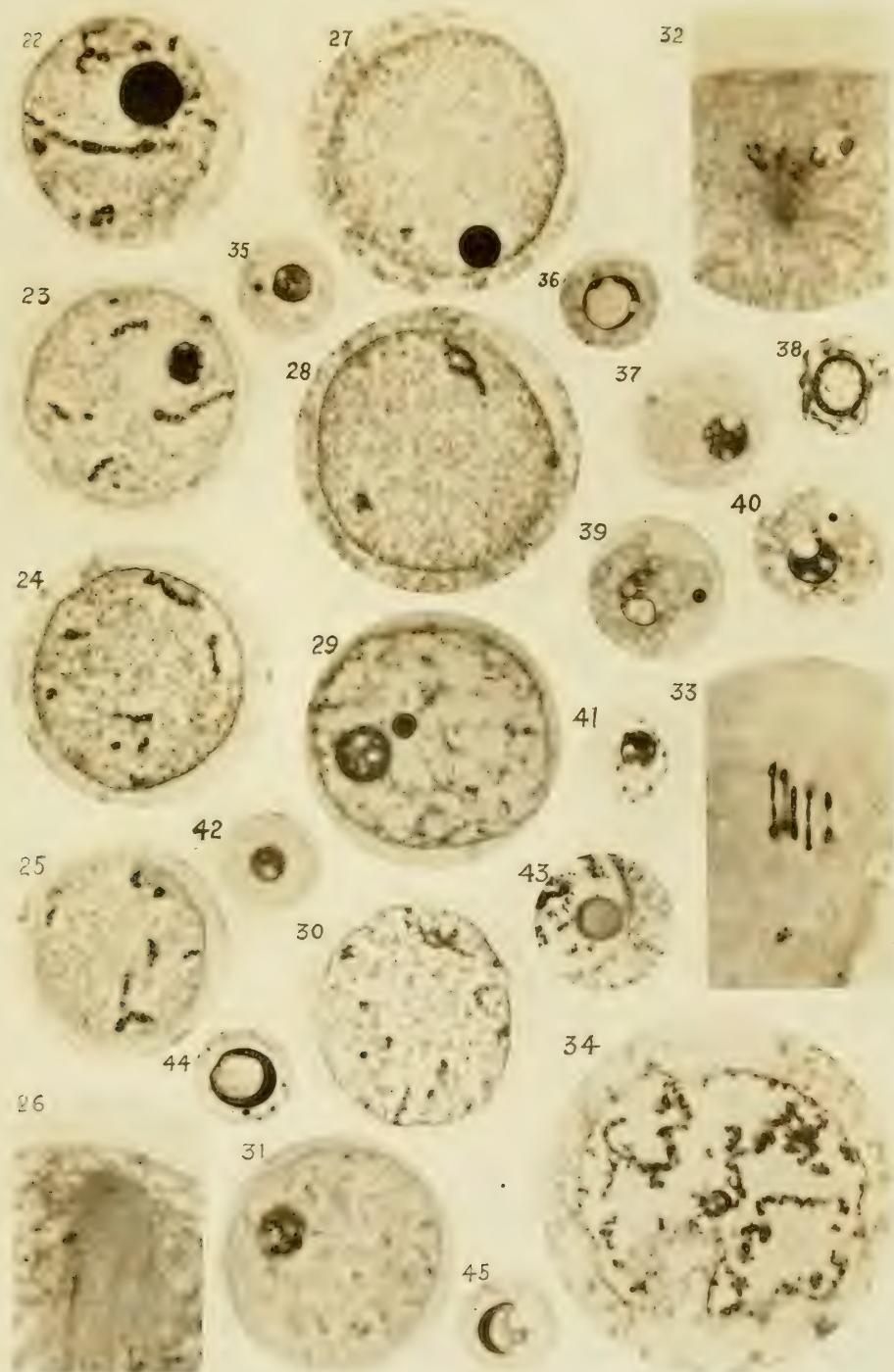
PLATE I.



PROPHASES OF FIRST MATURATION SPINDLE OF
ALLOLOBOPHORA FOETIDA.

FOOT & STROBELL PHOTOS.

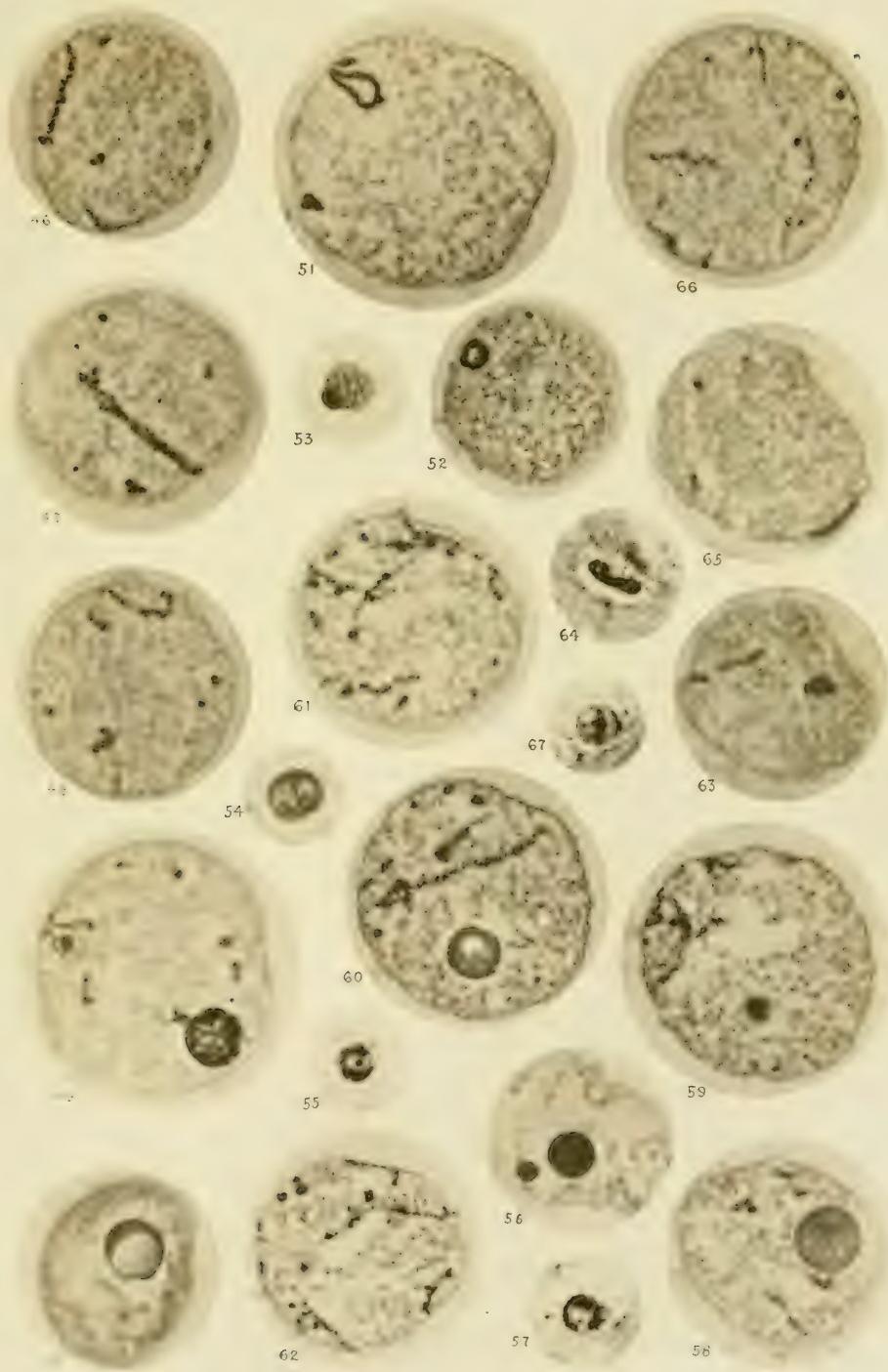
PLATE II



PROPHASES OF FIRST MATURATION SPINDLE OF
ALLOLOBOPHORA FOETIDA.

FOOT & STROBELL PHOTOS

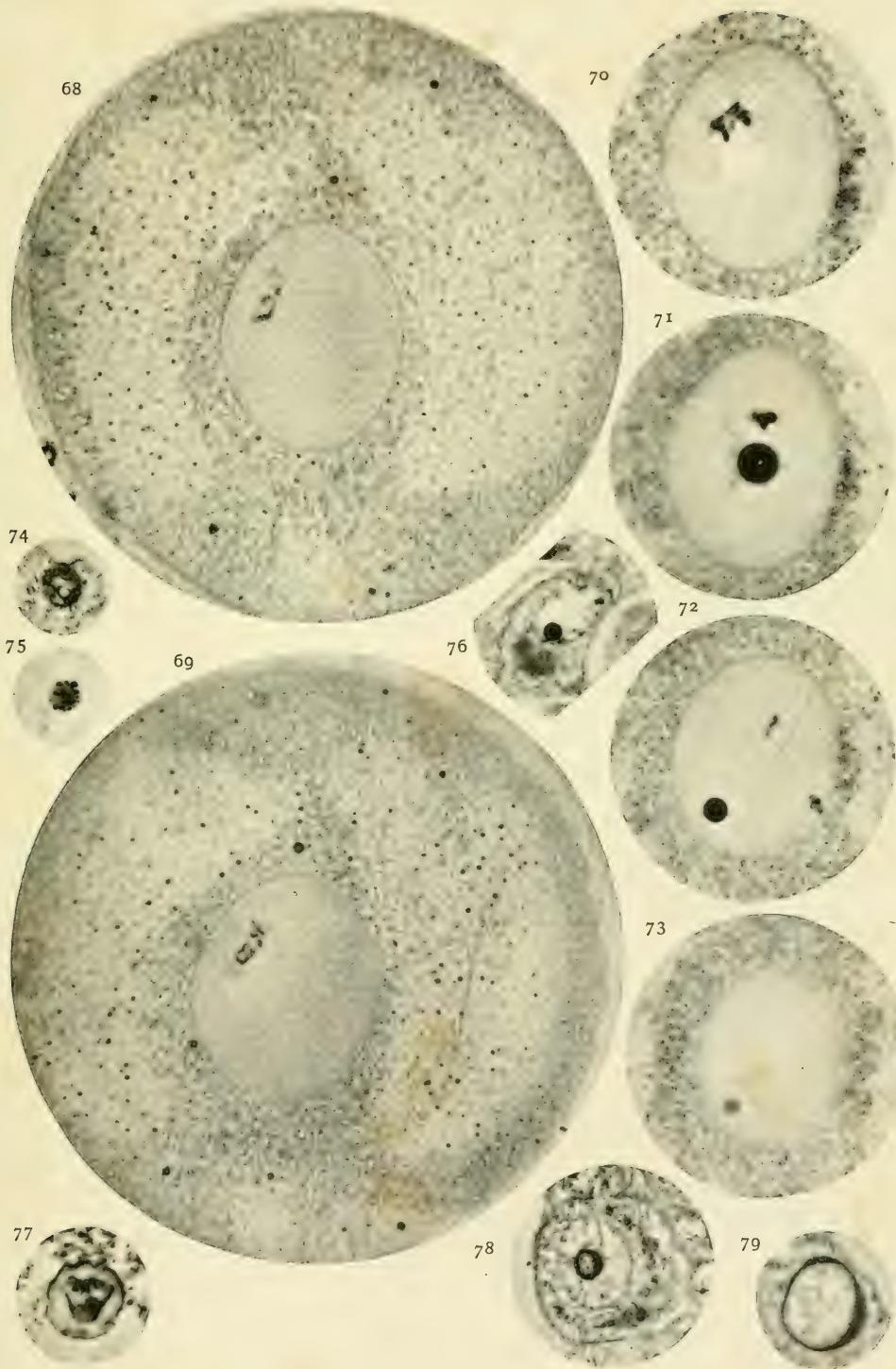
PLATE III.



PROPHASES OF FIRST MATURATION SPINDLE OF
ALLOLOBOPHORA FOETIDA.

FOOT & STROBELL PHOTOS.

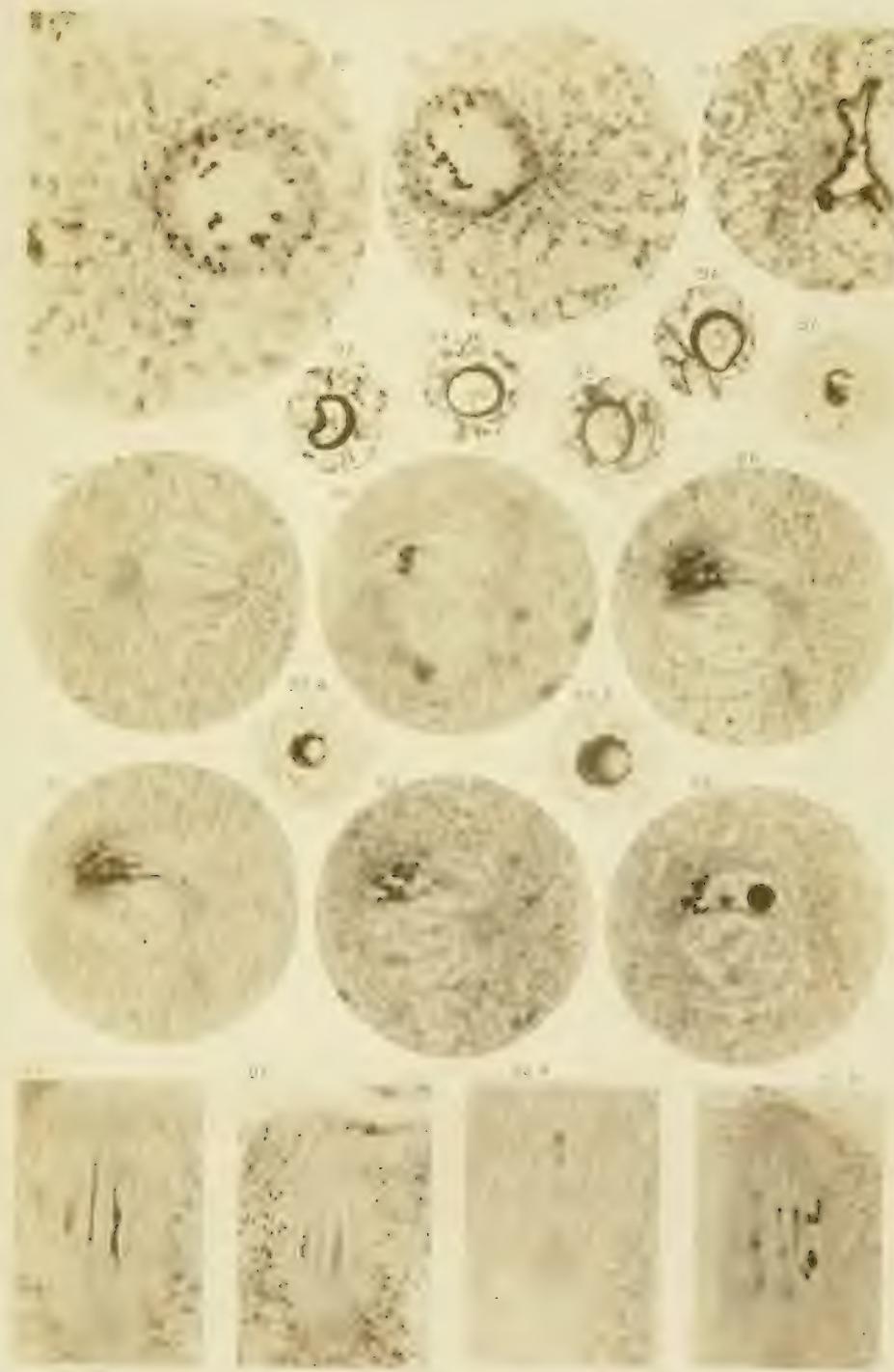
PLATE IV.



PROPHASES OF FIRST MATURATION SPINDLE OF
ALLOLOBOPHORA FOETIDA.

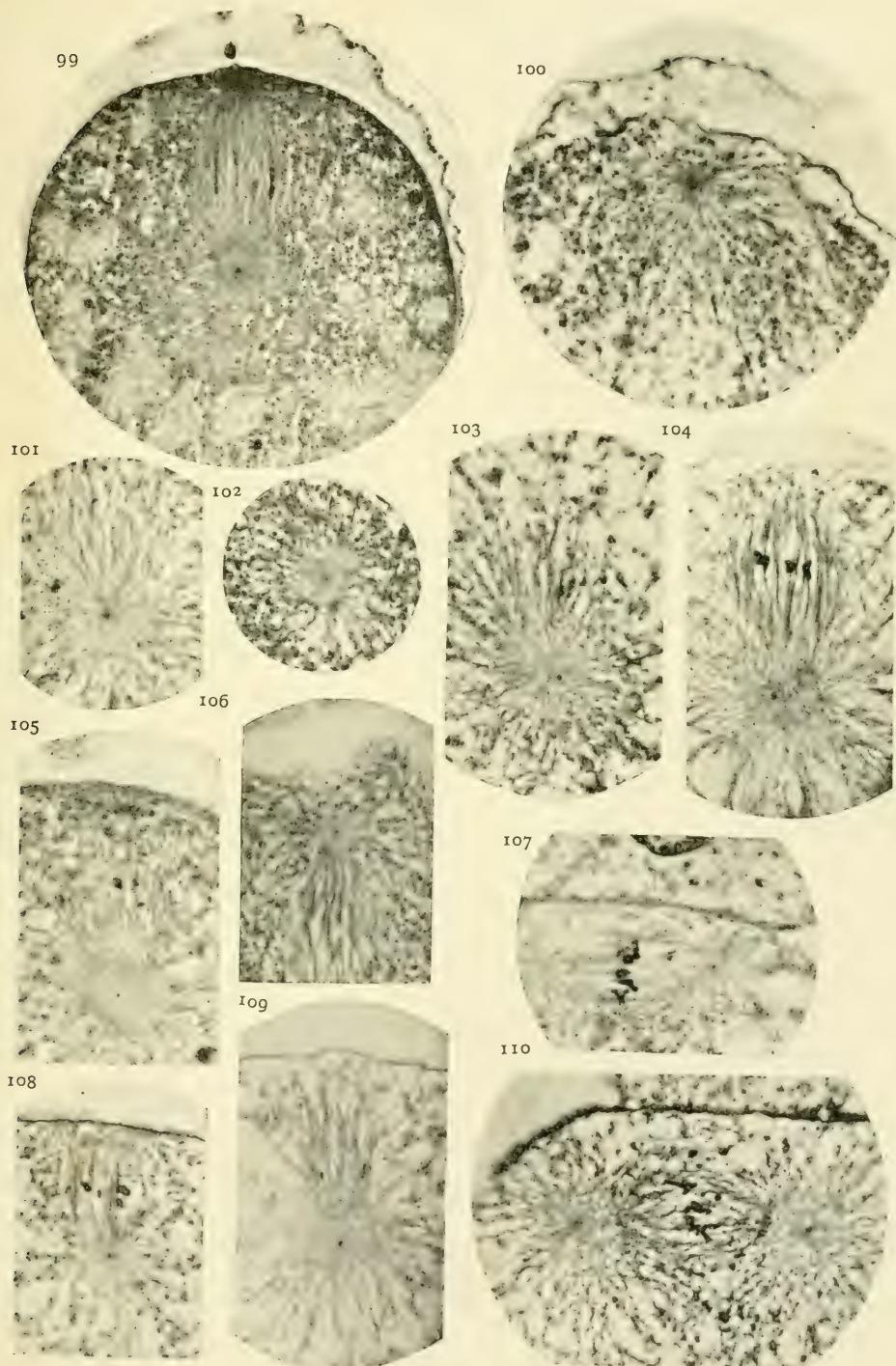
FOOT & STROBELL PHOTOS.

PLATE V.



PROPHASES OF FIRST MATURATION SPINDLE OF
ALLOLOBOPHORA FOETIDA. FOOT & STROBELL PHOTOS.

PLATE VI.



INOPHASES OF FIRST MATURATION SPINDLE OF
ALLOLOBOPHORA FOETIDA.

FOOT & STROBELL PHOTOS

PLATE VII



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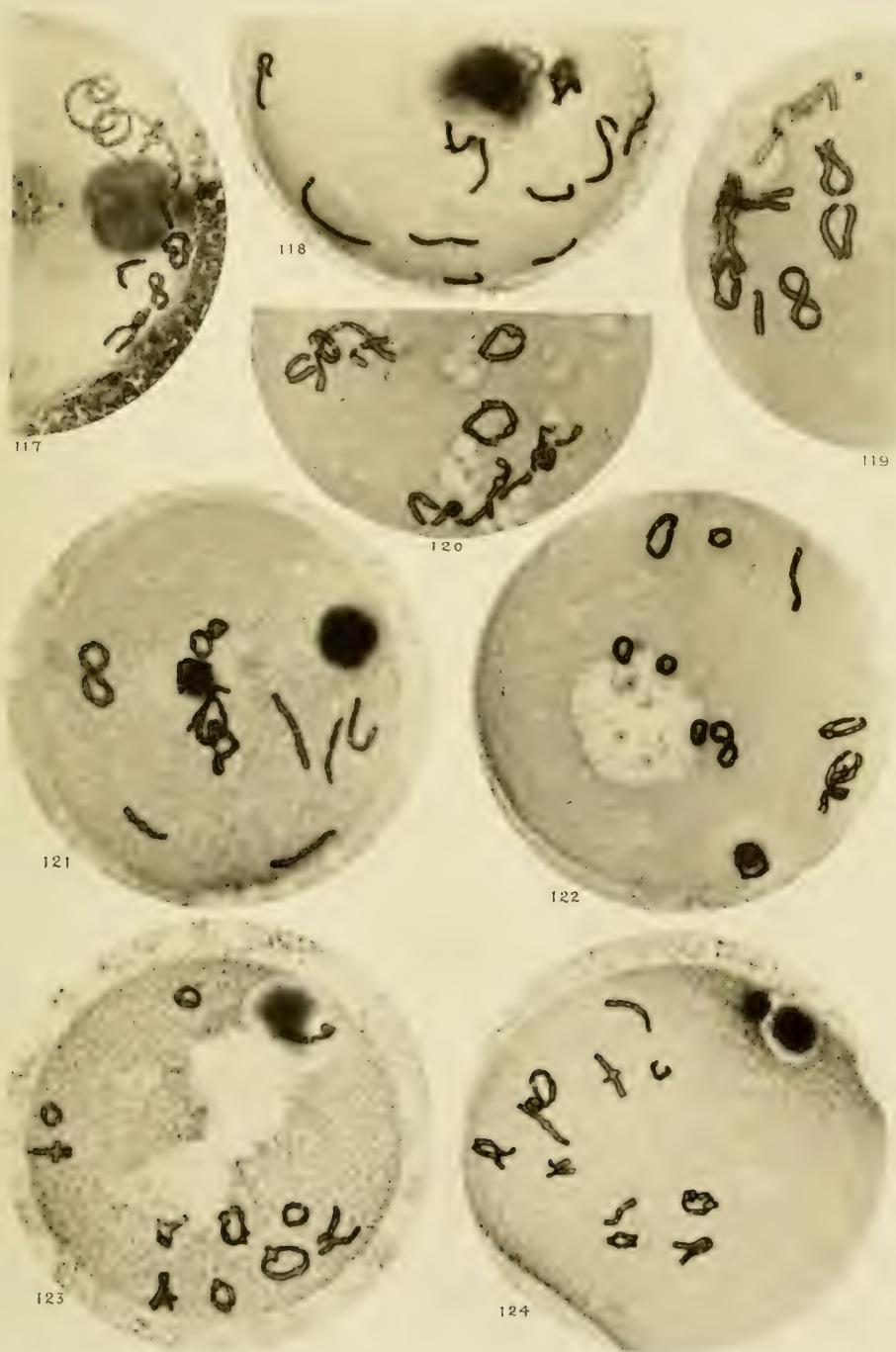


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PROPHASES OF FIRST MATURATION SPINDLE OF
ALLOLOBOPHORA FOETIDA.

FOOT & STROBELL PHOTOS

PLATE VIII.

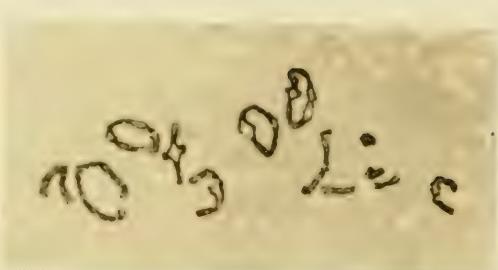


PROPHASES OF FIRST MATURATION SPINDLE OF
ALLOLOBOPHORA FOETIDA.
FOOT & STROBELL PHOTOS

PLATE I.



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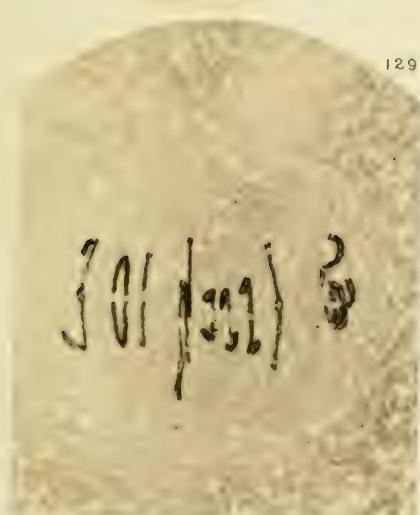


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GENETIC INTERPRETATIONS IN THE DOMAIN OF ANATOMY.*

PRESIDENTIAL ADDRESS BEFORE THE ASSOCIATION OF AMERICAN
ANATOMISTS.

BY

CHARLES-SEDGWICK MINOT, LL. D., D. Sc.

The science of anatomy, although one of the oldest of all sciences, was long neglected in America, and taught only in a routine fashion by professors who had little or no thought for the promotion of the science or any aim higher than teaching a certain number of established facts in gross anatomy to the maximum possible number of students. Within the last generation the few pioneers of anatomy have been succeeded by teachers, many of whom share the highest ideals of anatomical science, and have contributed important discoveries by which it has been really advanced. Our Society is at once the symbol and the outcome of these comparatively new conditions in America, and we have as our duty not only actively to encourage research, to spread anatomical knowledge, and to earn appreciation of anatomy as a living science, but also to exert a missionary influence by which the dignity and vitality of our science shall be brought to recognition at all our universities.

* The following recent or new technical terms are used in the course of the address and are recommended for adoption.

Cytogenic glands, false glands which produce cells, as for example, the lymph and genital glands.

Cytomorphosis, to designate comprehensively all the structural modifications which cells or successive generations of cells may undergo from the earliest undifferentiated stage to their final destruction.

False glands, all glands, which develop without ducts.

Lymphatum, a more or less definitely circumscribed area consisting of cellular reticulum, the meshes of which are charged with leucocytes and are in direct communication with lymph-vessels or more rarely with blood-vessels. It is a site for the multiplication of leucocytes.

Mesepatium, the membrane (French, *méso*) extending from the stomach and duodenum to the median line of the ventral abdominal wall, and in which the liver develops. It comprises a *dorsal mesepatium* (lesser omentum) and *ventral mesepatium* (falciform or suspensory ligament).

AMERICAN JOURNAL OF ANATOMY.—VOL. IV.

It is sometimes said, and perhaps more often thought, that anatomy is a completed science. This assertion is based upon the thoroughness and exhaustive character of the descriptions to be found in our textbooks of the anatomical conditions in the human adult; yet even as regards the organization of the adult we have still much to learn, especially concerning the microscopic structure with which we are still very imperfectly acquainted.

But anatomy is not alone a descriptive science. It is also comparative and genetic. In both these directions its development is very far from complete, and a vast amount of original research must still be completed before comparative anatomy and embryology shall have approached anywhere near even the present perfection of descriptive human anatomy.

To embryological research must be attributed a large part of the extraordinary progress which anatomy has made during the last twenty-five years. By embryology we have gained a far deeper understanding of all anatomical forms, we have acquired new interpretations for pathological facts, and we have secured for the first time some clear insight into the essential structure of the brain. I need not do more than allude to these achievements, since they are familiar to us all, and have most profoundly affected our anatomical conceptions. Our point of view has changed, and we interpret the anatomy of the adult in terms of the genesis of the organs and tissues during their embryonic development.

Perhaps no man has contributed so much towards this result as the great Leipzig anatomist, Wilhelm His, whose death this year we have to lament. He was a great master. He had full command over the problems of anatomy and contributed in the richest measure to their solution. His influence in America has been especially strong and widespread, and has certainly had much to do in bringing about the progress of anatomy in this country, which we are seeking to maintain, and if possible, increase. In what I have to say to you on this occasion, you will perceive

Phrenic area, the area on the superior or cephalad surface of the liver, by which the liver is attached permanently to the diaphragm. It includes the whole of the territory of the coronary and triangular "ligaments," so-called in current text-books.

Sinusoid, an irregular blood space, produced by the subdivision of a larger blood-vessel by the ingrowth of the parenchyma of an adjacent organ.

Structural unit, the territory of an organ supplied by a single terminal branch of an afferent vessel (artery or vein); the volume of such a unit is often only 10-20 cubic millimeters.

Trophoderm, the special layer of cells formed on the exterior of the young mammalian blastocyst, and serving to secure the implantation of the ovum in the uterus.

the influence of His clearly, and I cannot let the opportunity pass of expressing publicly my gratitude and admiration for the greatest anatonomist of his time.

Although embryology has already contributed in so ample measure to the promotion of our science, we are still far from having accepted all the enlightenment which she offers us. With your permission I will try to present to you certain embryological aspects of anatomy, the character of which I have sought to indicate in the title of this address, by the words "genetic interpretations."

First of all, let us consider the subject of cytomorphosis. This word I proposed in 1901¹ "to designate comprehensively all the structural modifications which cells or successive generations of cells may undergo from the earliest undifferentiated stage to their final destruction." As stated on that occasion it is convenient, though somewhat arbitrary, to distinguish four fundamental successive stages of cytomorphosis. These stages are (1) the undifferentiated stage; (2) the stage of progressive differentiation, which itself often comprises many successive stages; (3) the regressive stage or that during which degeneration or necrobiosis occurs; and (4) the stage of the removal of the dead material. The general data on which the conception of cytomorphosis is based have been briefly put together also in my text-book of embryology, and it seems therefore superfluous to dwell upon them at length in addressing you.

I cannot of course claim any greater originality in the establishment of the conception of cytomorphosis than is implied by the definite formulation of the ideas upon which it is based. These ideas have been gradually gathered as the fruit of numerous investigations in histogenesis. The mentioned investigations have made us all familiar with the conception of undifferentiated embryonic cells, with the gradual progress of differentiation in the cells during the embryonic, foetal, and even post-natal periods; and have also made us acquainted with various examples of degeneration and atrophy occurring in the course of development, both before and after birth. Up to the time when I proposed the term there had been, so far as I know, no attempt to survey all this array of facts from a single unifying point of view. But such a point of view is, I believe, well calculated to render our notions more precise as to many processes of development, and to afford us at the same time the practical benefit of being able to present the facts of histogenesis in our teaching in a way, which is very advantageous, because it facilitates in the student's mind the establishment of a real insight into the general course of development by emphasizing principles of very wide application. To me, at least, it seems that the conception of cytomorphosis should be made the

foundation of all our instruction in anatomy, and that its importance should be constantly emphasized in our class-rooms and that when good illustrations of cytomorphosis are encountered by the student, his attention should be especially directed to them, so that he may become familiar with the conception. Let me mention a few illustrations which I have found serviceable in teaching.

But first I must call your attention to an aspect of cytomorphosis, which has not hitherto, so far as my knowledge goes, been sufficiently emphasized. We may distinguish two fundamental phases. During the first, cell division occurs, during the second, cell division does not occur. During the first phase we may find a progressive alteration, which gradually takes place in successive generations of cells, but apparently the amount of differentiation which can occur while cells retain the power of active division is comparatively slight. During the second phase, since the cell no longer divides, the alteration takes place in the single cell, and the alteration, which occurs under these conditions, is typically great and may be best designated by the term final differentiation, differentiation being here held in our minds clearly distinct from degeneration. By final differentiation we mean the establishment of that special organization of a cell, which brings to perfection the specialized physiological function for which the cell is destined. Thus the alteration of a mesenchymal cell into a muscle fibre is its final differentiation, and establishes the physiological perfection of that cell as a contractile element. Beyond the final differentiation of the cell comes the series of degenerative changes. A comprehensive study of cell degeneration is yet to be made, nevertheless we can already say that, although cell degeneration is chiefly characteristic of the second phase of cytomorphosis, which is also characterized by the cessation of cell division, yet the degeneration may be initiated before the power of cell division is lost and the degenerative change in the cells may go on while they are still proliferating; but typically it seems rather that degeneration belongs to the second phase of cytomorphosis, and this seems to be alike true for necrosis and atrophy, that is to say, simple cell death, and for necrobiosis, that is to say, cell death preceded by structural changes, which we know commonly under the name of hypertrophic degeneration.

Let us pass on now to a few illustrations of cytomorphosis:

The first to which I would direct your attention is afforded by the formation of the "*trophoderm*." This is a new term which I have recently brought forward to designate the special layer of cells formed apparently from the ectoderm (or according to Assheton's theory, from

the entoderm) which serves to secure the implantation of the mammalian ovum in the walls of the uterus. In my "Text-Book of Embryology" I have figured these cells from the human ovum and applied to them the term trophoblast, but as Professor Hubrecht, who introduced this last term into science, has objected to this application of it, it has been necessary to introduce a new term, hence the designation trophoderm. It corresponds in large part, perhaps wholly, to that which Duval designated as the ectoplacenta. It is the first tissue in the mammals to be distinctly differentiated. The cells by their large size, distinct boundaries, and characteristic nuclei, are readily distinguished from any other cells existing in the embryo at the time the trophoderm is differentiated. Very soon after the development of the trophodermic cells, a large part of them begin to complete their cytomorphosis by undergoing degeneration and resorption. By their disappearance, as I have elsewhere pointed out, the intervillous spaces arise. The trophoderm therefore is not only the earliest tissue to be specialized in the development of mammals, but also the earliest tissue to absolutely complete its cytomorphosis.

Another striking illustration of the cytomorphic cycle with its phases of differentiation, degeneration, and disappearance of cells is offered to us by the blood corpuscles. The first blood corpuscles are cells with a minimum amount of protoplasm. The cells then proceed to grow, and as they grow, differentiate themselves in part at least, into red blood globules. In mammals there follows the stage, degenerative in character, by which the nucleus of these red blood corpuscles disappears. The manner of its disappearance is, to be sure, still perhaps a matter of debate, but for us for the moment is of minor importance. After the degeneration or disappearance of the nucleus, the blood corpuscles are destroyed and, having completed their cytomorphosis, are replaced by fresh ones.

A third admirable illustration is offered us by cartilage, and a fourth by bone. In cartilage we see at first a differentiation of simple mesenchymal cells which then enlarge, becoming the characteristic cartilage cells. When ossification of the cartilage occurs we can easily follow the hypertrophic degeneration of these same cartilage cells, which has been so much studied that good accounts of the enlargement and breaking down of these cells preliminary to the ingrowth of the osteogenetic tissue can be found in all the better text-books of histology; but I regret to say I do not recall any text-book either of anatomy, histology, or embryology, which points out the fact that this succession of changes in cartilage cells is a typical and almost perfect illustration of cytomorphosis. Almost the same can be said of bone, for in the formation of this tissue also we have first, the differentiation of the mesenchymal cells into osteoblasts, which

are always of larger dimensions than the cells from which they arise; and after these osteoblasts have become bone cells they cease their development and apparently degenerate. I have to say *apparently*, because, so far as I know, the fate of the bone corpuscle has not been ascertained with certainty. We risk but little, however, in asserting that the bone cells also offer an instance of a normal, complete cytomorphosis.

As a fifth and last illustration, let us choose the epidermis, in which we have a distinct type of differentiation. In the basal layer are the cells, which divide and produce, according to our present notions, all of the cells of the epidermis. When the basal layer cells divide, however, some of them only, pass immediately through further cytomorphic changes in order to make first, the cells of the mucous layer, and later, by undergoing cornification, to constitute the horny layer. Others of the basal cells remain members of the basal layer and continue to proliferate. We thus see the progeny of the original basal cells divided into two classes: the cells of one class pass on in their development, the others retain their ancestral type. In the epidermal cells we observe as in other instances of cytomorphosis, first the enlargement and differentiation of the cells, here occurring in the mucous layer, and later their degeneration or cornification followed by their necrosis and destruction.

It would be easy to multiply these illustrations. All of you could supply more. That which I would urge upon your consideration is the value of the cytomorphic interpretation in explaining the origin and differentiation of tissues in the light of the broadest principle of cellular development which we have up to the present time been able to establish.

I will now ask you to consider certain possible genetic classifications. The most fundamental and important of these seems to me to be that of tissues and of organs according to the germ layers from which they arise. This classification was made the basis of his entire course of lectures upon animal morphology by Professor Carl Semper, the Würzburg zoologist, under whom I had the pleasure of studying in 1875-76. It is not merely very practical and advantageous alike to teacher and pupil, but is also the only thoroughly scientific classification of structures and organs which we can adopt. No other classification should, in my judgment, be seriously considered. So firmly do I hold this conviction that I greatly deplore the fact that our text-books of histology are not written upon an embryological basis, the lack of which deprives them of much of the scientific character and value they ought to have.

As our knowledge of the development from the germ layers has grown, we have learned with ever-increasing certainty that each germ layer has its specific rôle to play. Each germ layer produces its own specific

set of tissues, which are not duplicated by the tissues of any other germ layer. I have already pointed out on another occasion that the importance of the germ layers is as absolute and unvarying in the domain of pathology as in normal differentiation; I need not dwell on that aspect of the question now, but will only repeat the declaration of my belief that the entire teaching of the pathologist as well as of the histologist and anatomist should be based on the doctrine of the germ layers and their specific rôles in histogenesis.

Almost any group of tissues would offer a favorable opportunity for the discussion of genetic classification. We may select those which are differentiated from the embryonic mesenchyma and which are commonly grouped in the adult under the names of the connective and supporting tissues. It is almost superfluous, so much is the genetic point of view neglected, to call your attention to the fact that in our current text-books of histology there is often little or nothing which would enable the student to grasp the relations of these tissues to one another or to understand their genetic relationships. It is true that our knowledge in spite of the great advances of recent years is still too incomplete to justify our asserting that the classification which we can now make is final. Nevertheless we can already perhaps attain approximate finality. A very great step in advance was made when the character of the cellular reticulum was established and it was shown that this tissue is different from ordinary connective tissue. It has two principal characteristics: first, the matrix or intercellular substance is nearly or absolutely fluid, so that leucocytes can wander freely in the intercellular spaces of the reticulum; second, the network of original protoplasmic filaments has become directly converted into a network preserving more or less the original form, but consisting not of protoplasm, but of a new chemical substance, reticulin. Where cellular reticulum is developed, as for instance in the so-called adenoid tissue, there may be formed from the cells a minimum amount of connective tissue fibrils and of elastic substance, but if we may judge from our present knowledge the cells, which have produced reticulin, preserve but a very small capacity for the production of other elements of connective tissue. Hence, it seems to me that we may well put cellular reticulum in a class by itself, quite apart from the true connective tissues in which the intercellular substance is not mainly fluid and in which there is an abundant development of fibrillar or elastic substance, or of both, and in which, further, reticulin is nearly if not wholly absent. We shall thus come to place all the connective tissues, properly so-called, in a second genetic group. When we follow in the embryo the history of young connective tissue, we learn that it undergoes two principal kinds of modifica-

tions, those affecting the matrix, and those affecting the cells. On these differences the classification in the following table is based.

We also know that connective tissue can be directly transformed into cartilage, which, therefore, unquestionably belongs in the same second genetic group as the true connective tissues. As regards bone, I find it somewhat difficult to reach a decision, but incline to the conclusion that bone should be regarded as distinct from the true connective tissue, thus making a third genetic division of the tissues derived from the primitive mesenchyma. This conclusion appeals to me partly as a protest against the absurd, though long established and honored, custom of separating cartilage from connective tissue, and putting cartilage and bone together in a common group under the head of supporting tissues. The following table presents the proposed classification in a form which you can easily follow:

TABLE I.—THE MESENCHYMAL TISSUES.

MESENCHYMA	Cellular reticulum	Matrix specialized.	Mucous tissue
	Embryonic connective tissue.....		Adult connective tissue
			Cartilage
	Bone	Cells specialized ...	Fat cells Pigment cells Smooth muscles Basement membranes Pseudo-endothelium Genital interstitial cells, etc.

Let me refer briefly to a third and more special example of the genetic classification of tissues, namely, that of the blood-vessels. As you probably all know, recent embryological investigations have compelled us to recognize not only the three familiar and long-known classes of blood-vessels, arteries, veins, and capillaries, but also a fourth class, that of the sinusoids. Capillaries arise as small vascular sprouts from pre-existing vessels, and these sprouts grow in the mesenchyma. A sinusoid, on the contrary, has an entirely different developmental history, for it is produced by the subdivision of a pre-existing and relatively large vessel. The subdivision is accomplished by the proliferating tubules (or trabeculae) of an organ, which encounter a large vessel and invade its lumen, pushing the endothelium before them.⁴ The endothelium of the vessel, on the other hand, expands and spreads over the tubules (or trabeculae). By the convolutions and anastomoses thus produced, a large vessel is subdivided into small ones. It follows that a sinusoidal circulation is purely venous or purely arterial. It may suffice, upon this occasion, to point out again that the structure of many important organs, as for

example, the liver and Wolffian body, cannot be understood or even described correctly without taking into consideration the sinusoidal character of their circulation. In this case also, the adoption of the genetic interpretation is much needed. We shall apply presently the concept "sinusoid" to aid us in the interpretation of glands.

We may pass now from a consideration of tissues to that of organs, and begin with the glands. The classification, which at the present time prevails widely, is one based upon certain incidental peculiarities in the shape of the secretory portion of the glands, so that they are put into two main divisions: the tubular and alveolar; then under each of these we have three main parallel groups:

- the simple tubular or alveolar glands;
- the simple branched tubular or alveolar glands;
- the compound branched tubular or alveolar glands.

But in this classification there is no place for such a gland as the liver and the thyroid. In text-books of histology, we find the liver tucked in under the tubular glands and designated as a reticulated tubular gland, and the thyroid placed as a follicular gland under the head of the alveolar. But in another way the system also fails, for there are tubulo-alveolar glands which again must be classified as simple, simple branched, or compound branched. Essentially this classification is adopted by the authors of the manuals of histology which I have examined. To classify glands thus, seems to me about on a par with classifying organs by their being solid or hollow, a principle, which would put the spinal cord in the same class with the intestine, and nerves in the same class with the tendons. The peculiarities of shape of the secretary portions of glands are entirely secondary, and do not indicate anything fundamental in regard to the structure of the gland itself. We cannot call a system good which, if applied in accordance with its own definitions, would put some of the mucous glands of the stomach in one division, others in another division, because, although these glands are alike in their histological structure, some of them are branched and some are not. Must we not condemn a view, which excludes the ovary from the glands and makes the testis a compound tubular gland, although ovary and testis are strictly homologous organs, even in the details of their structure? These are only samples of the innumerable difficulties which the system encounters, because it is essentially pedantic, admirable as an orderly arrangement of names, but impossible as a presentation of anatomical facts.

It appears to me not difficult to make an entirely new classification of

glands, which shall be based upon their genesis and upon the morphological distinctions, which exist between them. To begin with, we may put the unicellular glands, of which the goblet-cells serve as the most familiar type known in man, in the Class A, v. p. 256; next we may have the true multicellular glands of the epithelial type, which always develop with ducts by which their secretion is discharged; these form Class B; while a third class would include the false glands which never develop with ducts, which produce either merely an internal secretion so-called, or are adapted to the development of cells of special kinds, as, for example, the lymph- and genital-glands; such structures constitute Class C.

We must first attempt a classification more in detail of the true epithelial glands (Class B). In my opinion we can best make two fundamental divisions. The glands of the first division have often been called single or simple follicular glands; I propose for them the term "*simple glands.*" The glands in question are always small and have one or several centers of growth according as they are simple tubes or slightly branching. Those of each kind are always very numerous and they occur more or less near together over considerable areas. There are two types of these known. The first are simple invaginated areas with scattered unicellular glands, as for instance the glands of the large intestine, the so-called Lieberkühn's follicles; they might be called simple follicles. Glands of the second type are invaginated areas with specialization of the cells, as, for example, the sweat, gastric, and sebaceous glands; they might be called glandular follicles. In the accompanying table the principal glands of this division are enumerated.

The glands of the second division are of greater bulk and are often referred to as organic or branching glands. I propose to name them "*compound glands.*" They are provided with a single main duct, which is more or less freely branched, each branch connecting finally with the secretory portion proper of the organ, which portion may itself also be branched or not. Each gland falling in this division is a more or less complete organ by itself, receiving its special blood supply, and its special innervation—is, in short, a clearly marked physiological entity. Such a gland differs profoundly in its plan of organization from the glands of our first division. Of the second division there are clearly three main types to be distinguished. In the first type the branches of the glands are found to be supported by mesenchyma or its derivative, connective tissue, which is more or less abundant between the ducts and secretory elements of the organ, and in the mesenchyma there is a capillary circulation, which is often brought, however, into intimate proximity with the epithelial elements of the organ. These organs are further character-

ized by the fact that their branches remain distinct. In the second type, on the other hand, the branches unite together and form an anastomosing gland structure, and when this anastomosing condition is found it is associated, not with the development of connective tissue and capillaries, between the epithelial elements of the organ, but, on the contrary, with the presence of a sinusoidal circulation. The branching glands with capillary circulation are numerous, and they may arise, as is noted in the table, from either the ectoderm, the entoderm, or the mesothelium. Glands of the second type, anastomosing and furnished with sinusoids, are few in number. The liver is, of course, the most typical and the most important. With the liver we ought perhaps to associate the parathyroid, for the recent and still unpublished investigations of Dr. John Warren show that when this gland is highly developed it is of an anastomosing type, and make it probable that its blood supply is sinusoidal.

There remains still a third type, which is necessary, because the ducts become obliterated in a certain number of true epithelial glands, which develop primarily with ducts. There results in each case a group of hollow epithelial follicles, which are characteristic. For this type I propose the name, "*ductless epithelial glands*." The thyroid gland and the hypophysis are probably the best-known illustrations of this group of glands. Although the morphology of the pineal gland (epiphysis) is obscure, the organ seems at present to belong to our third type.

Our third Class, C, comprises the false glands, which never develop with ducts. So far as I am aware this statement may be made absolute for all glands of this class. It is true beyond any possible question for most of the glands, which are here to be considered, but it is perhaps as well to note that possibly some of the glands of the first division may be found in some vertebrates to have been primitively provided with ducts. This seems to me possible, but not probable. The first division of the false glands are the epithelioid. They are perhaps exclusively, so far as the essential gland elements are concerned, of entodermal origin, and it has become probable that their circulation is typically sinusoidal. In the present state of our knowledge it would be venturesome to make positive assertions on these two points. In the epithelioid glands we have groups of cells of epithelial origin separated, in the adult at least, from the layer which produced them, and brought into intimate relation with blood-vessels. A second division comprises the mesenchymal ductless glands, which are similar to the epithelioid glands in their general appearance, but their specific elements are derived from the middle germ layer. As an illustration of the ductless false gland of the first division, I may mention the parathyroid; of the second division, the suprarenal cortex. As

to the position of the thymus, I feel quite uncertain and hardly dare to say whether it should be placed among the epithelioid glands or among the cell-producing glands. Similarly, how to place the interstitial cells of the genital glands in our system is not yet quite clear to me. The third division is that of the cytogenic glands, and of these we may readily distinguish three important types: the first, those in which lymph cells arise; second, those which produce red blood corpuscles; and third, those which yield the genital elements. The glands of the first type may be called lymphæal structures. "Lymphæal" is a new term derived from "Lymphæum," itself a new technical expression, which I have used for several years in my lectures on histology and have found advantageous. A lymphæum may be defined as follows: it is a site for the multiplication of leucocytes and is a more or less definitely circumscribed area consisting of cellular reticulum, the meshes of which are charged with leucocytes and are in direct communication with lymph-vessels, or more rarely with blood-vessels. The following offer examples of lymphæa: solitary follicles, tonsils, thymus, lymph glands, hæmolymph glands and spleen. As stated above, whether the thymus should belong in the first or third division, I cannot say. Of the second type in this division, the bone marrow is the most important example. Of the third type, that of the genital glands, we have of course to distinguish two forms, the ovary and the testis.

With these explanations, I hope the accompanying table will be clear and I trust that the proposed new classification of glands will seem to you both more scientific and more available than the classification now prevalent, which I should like to see displaced.

TABLE II. CLASSIFICATION OF GLANDS.²

CLASS A. *Unicellular.*

CLASS B. *True Glands*, always developed with ducts.

DIVISION 1. *Simple Glands, (unifollicular or single glands).*

a. Ectodermal.

1. Sweat glands.
2. Sebaceous glands.
3. Buccal glands.

b. Entodermal.

1. Oesophageal.
2. Gastric.
3. Intestinal.

c. Mesothelial.

1. Uterine.

DIVISION 2. Compound Glands (*organic or true compound glands*).

Type a, ductless epithelial branching (*with capillary circulation*).

1. Ectodermal.
Salivaries, tear gland, Harderian.
Mammary glands.
2. Entodermal.
Pancreas.
3. Mesothelial.
Appendicular glands of the urogenital system.

Type b, anastomosing (*with sinusoidal circulation*).

1. Liver.
2. Paraphysis (in *Necturus*).

Type c, ductless epithelial (*with secondary obliteration of duct*).

1. Thyroid.
2. Hypophysal gland.
3. Infundibular gland.
4. Pineal (*epiphysis*).

CLASS C. False Glands, never developed with ducts.**DIVISION 1. Epithelioid glands (*exclusively entodermal?*)**

1. Parathyroid.
2. Carotid.
3. Thymus (*cf. below*) (?).

DIVISION 2. Mesenchymal ductless glands.

1. Suprarenal cortex.
2. Coccygeal gland and other chromaffinic cell organs.
3. Interstitial cells of genital glands (?).

DIVISION 3. Cytogenic glands.

- a. Lymphæal structures.
 1. Lymph glands and follicles (tonsil?).
 2. Hæmolymph glands.
 3. Spleen.
 4. Thymus (?).
- b. Sanguifactive organs.
 1. Bone marrow.
- c. Genital glands.
 1. Ovary.
 2. Testis.

I should like to include, in passing, reference to another general anatomical conception which, though not based strictly on embryological results, may be appropriately mentioned. I mean that unit of adult organization, which is sometimes referred to as the "*lobule*," but, as this term is somewhat confusing owing to the manifold meanings assigned to it, I venture to express the hope that the term "*structural unit*" will be

used instead, as has already been done by a few writers. We can then continue to employ the term lobule for the lung and the liver in the senses tradition gives to the term, as used for these two organs, and avoid confusion. The *structural unit*³ may be defined as the territory of an organ supplied by a single terminal branch of an afferent vessel (artery or vein). The volume of such a unit is often only 10-20 cubic millimeters. In the case of the liver, the structural unit comprises parts of several adjacent so-called lobules. It is a pleasure to recall that the recognition of the anatomical importance of these units is due to one of our most distinguished American investigators, Dr. Mall.

Finally, I should like to apply the principle of genetic interpretation to descriptive anatomy. It will, I think, sufficiently expound the point of view I am advocating to consider the application of the principle to a single organ, and for this purpose we may conveniently select the liver. In order to show that what I propose is practically a real and great innovation, let me indicate to you briefly the character of the anatomical descriptions of the liver to be found in some of the leading text-books of human anatomy.

In Cunningham's Anatomy (1902), the account of the liver is written by Professor Birmingham. He describes, 1, the general form of the surface; 2, the topographical relations and surfaces in detail; 3, the fissures, without giving their morphological relations; 4, the division into right and left chief lobes; 5, the peritoneal relations and ligaments; 6, the physical characteristics.

In the tenth edition of Quain's Anatomy (1896), the description opens, 1, with the dimensions and weight; 2, the surfaces; 3, the fissures; 4, the ligaments and the omentum; 5, the topographical relations; 6, vessels and nerves; 7, the ducts.

Testut in the third volume of his Anatomy (1894) gives, 1, the situation; 2, fixation; 3, volume and weight; 4, general confirmation, including the two chief right and left lobes; 5, the surfaces in detail.

The account of the liver in Poirier's Anatomy, Volume IV (1895), is written by Charpy, who begins with 1, the definition, and continues with 2, situation; 3, fixation; 4, data as to weight, consistency, etc.; 5, the form and surfaces. Under the head of fixation Charpy says:

“ La foie est suspendu à la voûte du diaphragme par deux moyens d'attachement : par des replis péritonéaux et par la veine cave inférieure.”

This misleading statement is the more deplorable because he mentions only incidentally that the liver adheres directly to the diaphragm. Quite at the end, the division into the right and left lobes is mentioned.

In the fourth edition (1901) of Merkel-Henle's *Grundriss*, there is, 1, a general account, which is distinctly not morphological in character; 2, detailed description of the surfaces and topography; 3, of the histology.

Gegenbaur in the seventh edition of his Anatomy (1899) proceeds very differently, for he has strong morphological inclinations. He gives, 1, the general account of the development of the liver; 2, general account of the surfaces, including the division into the chief lobes; 3, the relation of the veins to the omentum and the falciform ligament. Gegenbaur is the only author of a text-book of human anatomy, known to me, who gives a distinctly morphological account of the human liver, but even his presentation of the subject leaves much to be desired, chiefly because his knowledge of embryology was meagre, and quite insufficient for an adequate interpretation.

It would be easy to analyze descriptions in other text-books, but enough has been presented to show that they are usually characterized by certain common tendencies. The authors dwell upon the position and shape of the liver, seeking to emphasize its exact form, but not endeavoring at all to emphasize the essential characteristics of the organ, or to bring out the significance of its parts in a manner satisfactory to either an embryologist, a physiologist, a morphologist, or a pathologist. With the exception of Gegenbaur, none of the accounts rises above the level of sheer description.⁵ They simply perpetuate the tradition inherited from the time when human anatomy was only the description of what was actually found in the human adult. That tradition has undoubtedly been in part maintained by the demands of surgeons, whose interest is necessarily chiefly given to the exact determination of the topographical divisions in the body, hence the influence of the surgeons, when dominant in the anatomical laboratory, has often exerted an influence unfavorable to the becoming maintenance of a scientific spirit, such as we ought to insist upon for the sake alike of anatomy and medicine.

If we review collectively the brief analyses just given of the actual descriptions in the text-books, we realize at once that those points, which the genesis of the liver reveals to us as fundamental, are scarcely heeded by the authors whom we have reviewed. This is not a fitting occasion to attempt a new description of the liver, and I can merely indicate to you the principal points upon which a scientific description ought, in my opinion, to be based. No little study and care would be necessary to work out practically the suggestions, embodied in the following schedule. Indeed, the schedule can doubtless be improved by others.

In order to prepare an adequate description of the liver, we must begin by laying aside certain bad habits which we have inherited and have

allowed ourselves to perpetuate. I mean the habit of applying the term ligaments, and the habit of applying the term fissures, to the liver; also the habit of describing the hepatic segment of the vena cava inferior as a vessel distinct from the liver, it being in reality, strictly, in every sense of the word, a portion of the organ. It may be further suggested that the introduction of a new term, *mesepatium*, may assist in clarifying the relations. The "*mesepatium*" is the membrane (French *méso*), which stretches from the ventral border of the stomach and duodenum to the median line of the ventral abdominal wall. It is in this membrane that the liver develops. Above the liver, between it and the stomach, is the dorsal mesepatium (lesser omentum). Between the liver and the body wall is the ventral mesepatium (falciform or suspensory ligament). Instead of speaking of the ligaments, we should speak of the insertion of the dorsal and ventral mesepatium into the liver; and instead of coronary and triangular ligaments, we should speak of the attachment of the liver to the diaphragm. This area of attachment might be called, as regards the diaphragm, the hepatic area, as regards the liver, the phrenic area.

With these preliminary explanations in mind, it may be suggested that a description of the liver must begin, as many authors have begun it already, with a general statement in regard to the position, size, color, and general form of the organ, and explaining that it is a gland, with a duct opening into the duodenum, and having the gall bladder appended to it, and that the circulation is sinusoidal, and not capillary.

Next, I should place a careful statement of the fundamental relations, as follows, *first*, of the broad connection of the liver with the diaphragm. This connection is primitive embryologically, is maintained throughout life and constitutes the phrenic area. It is not by the so-called ligaments or peritoneal folds, nor is it by the vena cava inferior that the liver is attached to the diaphragm. On the contrary it is by a large and characteristically shaped phrenic area of the organ that the connection is established. *Second*, the relation of the liver to the mesepatium, pointing out especially that the insertions of the dorsal and ventral mesepatia mark the division of the liver into right and left lobes and that the insertion is enlarged at one point towards the right to form the so-called porta of the liver, which admits from the dorsal mesepatium the hepatic artery, bile duct, and portal vein. *Third*, the relation of the veins to the organ, emphasizing that the portal vein marks the border of the dorsal mesepatium, and that its branches within the organ mark the so-called portal canals; emphasizing also that the umbilical vein or venous ligament marks the free edge of the ventral mesepatium, and explains the position,

origin and adult state of the ductus venosus. *Fourth*, the entrance and exit of the vena cava inferior. In this connection there should be made clear the rôle of the caval mesentery in furnishing a path for the *cava inferior*, and at the same time shutting off the lesser peritoneal space, and keeping the surface of the Spigelian lobe as part of the boundary of this space.

Next, again, might be presented the secondary features, especially the marking off of the caudate lobe from the chief right lobe by the vena cava inferior, and the marking off, similarly, of the quadrate lobe by the porta and the gall bladder.

Finally, according to this schedule, the description of the finer surface modelling and the contact with various adjacent organs, such as the stomach, colon, duodenum and kidney. Not one of these topographical relations is indispensable for a comprehension of the general character of the organ. Even from the standpoint of the surgeon and physician they are of minor importance. If they are put, as has been customary, in the forefront of text-book descriptions, attention is distracted from more essential things. Surely one need not argue to prove that a general comprehension of each organ is, first and last, the most important goal, to be striven for in the study of it.

In regard to almost every organ in the body it may be said, I think without injustice, that the current anatomical text-books offer bare and barren form-descriptions, seldom giving much, and often giving no, consideration to the essential morphological features of the parts. Take, for example, the urogenital system. We all know that the internal female genitalia are formed of two urogenital ridges, which fuse in the median line, making the so-called genital cord. There is in each ridge a longitudinal epithelial duct, which becomes the Fallopian tube, and by fusing with its fellow in the genital cord, produces the cavity of the uterus and vagina. A projection on one side of each ridge forms the ovary. Where the ridges have not united, rudiments of the Wolffian body of the embryo occur. The surface of the ridges, both where they are separate and where they are united, is covered by mesothelium. Around the duct (Fallopian tube), there is developed a muscular layer, and around the uterine portion of the fused ducts in the female a very powerful musculature is developed. By the union of the two ridges a partition is formed across the pelvic end of the abdomen, so that the abdominal cavity forms a pocket on the dorsal, and another on the ventral, side of the genitalia. Now the anatomical way of describing these organs is not to mention the ridges at all, but in the case of the female to speak of the uterus and its liga-

ments. It seems sometimes as if a deliberate effort were made by the descriptive anatominist to exclude all liberal use of the understanding and of the intelligence from the study of anatomy, and to reduce it almost to mere memorization of shapes and proportions, exceedingly difficult to fix in the mind by that method.

Is one not justified in condemning with great severity the perpetuation of this old type of anatomy? Is it not a grave mistake to fail to take advantage of the progress of anatomical science, and to utilize the best results of anatomical investigation to aid us in forming for ourselves, and still more, perhaps, for our students, clear notions of the essential characteristics of human organization? There has been within the last twenty-five years a very great progress in our knowledge of the topographical anatomy of the viscera, both thoracic and abdominal. When I plead for the presentation of the subject from the genetic standpoint, I do not mean to imply that this superior topographical knowledge should be slighted, but, on the contrary, I believe that if the student can first master the essential morphological relations of the body, it will be easier for him to master subsequently the finer, and often practically important, topographical details. Let our motto be, not "to memorize," but "*to comprehend*" the facts of anatomy.

Embryology illuminates anatomy. Its teachings give us the intellectual mastery of anatomical science, because embryology analyzes details, discriminates the essential from the secondary facts, and establishes the genetic interpretation, in the solvent light of which the obscurities of ancient anatomy vanish, and we see, where before was a dead sea of innumerable facts, new vital laws arising and guiding principles.

NOTES.

1. P. 247. Cytomorphosis was first used in the Middleton Goldsmith Lecture for 1901, entitled "The Embryological Basis of Pathology," *Science*, XIII, 481.

2. P. 256. Perhaps all or some of the salivary glands are entodermal. The submaxillary gland belongs among the organs, when it is a single large compound gland with a Bartholini's duct. When the submaxillary is represented by a group of small glands, they belong with the other simple buccal glands.

The position of the mammary gland must remain uncertain, until we can decide whether it is merely a group of glands, or morphologically a true compound gland. The significance of its peculiar development is still unsettled.

The hypophysis will perhaps, with more accurate study, be found to be an anastomosing gland with a sinusoidal circulation.

3. P. 258. The morphological characteristics of the structural (or histological) unit have been pointed out by Mall, so that the brief inadequate definition seems sufficient for the occasion.

4. P. 252. The account of the formation of sinusoids is somewhat schematic. We now know that the intercrescence of the vessels and parenchyma offers variations especially in its mode of beginning.

5. P. 259. Huntington's Anatomy of the Peritonæum, etc. (1903), is written entirely from the genetic and comparative standpoint. This excellent work, however, is not a general text-book, and in no sense belongs in the class of manuals criticized in the text. Even Huntington's account of the liver seems to me not to take sufficient advantage of our morphological knowledge, especially as regards the primary connection of the liver with the diaphragm and also as regards the sinusoidal circulation.

STUDIES OF THE DEVELOPMENT OF THE HUMAN SKELETON.

- (A). THE DEVELOPMENT OF THE LUMBAR, SACRAL AND COCCYGEAL VERTEBRAE.
- (B). THE CURVES AND THE PROPORTIONATE REGIONAL LENGTHS OF THE SPINAL COLUMN DURING THE FIRST THREE MONTHS OF EMBRYONIC DEVELOPMENT.
- (C). THE DEVELOPMENT OF THE SKELETON OF THE POSTERIOR LIMB.

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WITH 13 PLATES.

The following studies on skeletal development are based upon embryos belonging to the collection of Prof. Mall, at the Johns Hopkins University, Baltimore. I am greatly indebted to Prof. Mall for their use.

A.

THE DEVELOPMENT OF THE LUMBAR, SACRAL AND COCCYGEAL VERTEBRAE.

Recently I have given an account of the development of the thoracic vertebrae in man (This Journal, Vol. IV, No. 2, pp. 163-175). In the present paper it is my purpose to describe the special features which characterize the differentiation of the more distal vertebrae.

During the early stages of vertebral development the skeletal apparatus of the various spinal segments is strikingly similar. This is shown in Fig. 1, Plate I, which illustrates the spinal column of Embryo II, length 7 mm. Yet even during the blastemal stage some regional differentiation becomes visible. The costal processes of the thoracic vertebrae, for instance, develop much more freely than those of other regions. It is, however, during the chondrogenous period that the chief regional features appear.

To what extent morphological similarity in the sclerotomes and scleromeres indicates equal formative potentiality experiment alone can determine.

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mine. While it is unlikely that experimental studies of the required nature can ever be made on mammalian embryos it is quite possible that they may on embryos of some of the lower vertebrates. From the evidence at hand it seems probable, however, that the primitive vertebræ are to a considerable extent potentially equivalent and that their subsequent development depends upon the demands of their regional environment. The strongest arguments in favor of this view come from a study of variation in the adult. It is well known that at the regional boundaries vertebral variation is frequent. Thus the 7th vertebra often carries a short "cervical" rib (Gruber, 69), and rarely it has two cervical ribs which run to the sternum (Bolk, 91). On the other hand the 8th vertebra, usually the first thoracic, may assume all the characteristics of a cervical vertebra (Leboucq, 98, Low, 91).

At the thoracico-lumbar margin variation is more frequent than at the cervico-thoracic. Thus out of 1059 instances described statistically in the literature I found, 1904, that the 19th vertebra, commonly the last thoracic, had no free ribs and was hence of the lumbar type in 30 instances, 2.8%, and that on the other hand the 20th vertebra, commonly the first lumbar, had free costal processes in 23 instances, 2.2%. Cases have also been reported where the 21st vertebra, usually the 2d lumbar, has carried free ribs (Rosenberg, 99). Variation takes place in the articular processes as well as in the costal elements of the vertebræ at the thoracico-lumbar border (Topinard, 77).

Variation of the lumbo-sacral boundary is likewise frequent. Thus out of the 1059 instances above mentioned in 28 instances, 2.7%, the 24th vertebra, commonly the 5th lumbar, was the first sacral and in 47 instances, 4.4%, the 25th vertebra was of the lumbar type. The 25th vertebra may exhibit one of many transitions from the sacral to the lumbar type. This subject has been well treated by Paterson, 93. Papillault, 90, has contributed an interesting paper on lumbar variations and Cunningham, 89, on the proportion of bone and cartilage in the lumbar region.

At the sacro-coccygeal border variation is even more frequent than in the regions more anterior. Paterson, 93, found diminution in the number of sacral vertebræ in 2.62%, and increase in their number in 35.46% of the 265 sacra he examined; and Bianchi, 95, in 17.5% of the female, 23.3% of the male, and 21.23% of the total number (146) of sacra examined. In this count he excluded sacra in which compensation for lumbo-sacral alterations was to be seen. Bianchi thinks that the 1st coccygeal vertebra belongs properly to the sacrum. In the 1059 instances mentioned above I find that the 30th vertebra, usually the 1st coccygeal,

was reported sacral in nature in 91 instances, 8.6%, and the 29th, commonly the last sacral, coccygeal in 27 instances, 2.5%. It is possible that variations of this nature were sometimes overlooked by those making up the tables from which the above data were obtained.

Variation other than border variation has been reported most frequently in the cervical region. Ribs have been found not only on the 7th and 6th vertebrae but also on those more anterior (Szawłowski, 91).

It seems fair, however, to assume that the primitive vertebrae become differentiated according to the demands of their environment. Thus the factors commonly exerted on the 8th to 19th costo-vertebral fundaments causing them to develop into thoracic vertebrae with free ribs, may be so exerted as to call into similar development the 7th to 18th, the 7th to 19th (20th), the 8th to the 18th, the 8th to the 20th (21st), or the 9th to the 19th (20th). While the thorax may be segmentally extended or reduced at either end, extended at both ends, or extended at one end and reduced at the other, a simultaneous reduction at both ends has not been reported (Rosenberg, 99).

Differentiation in the post-thoracic region depends apparently in the main upon the position of the posterior limb (Bardeen, 90, Ancel and Sencert, 92). When the developing ilium becomes attached to the costal processes of the 25th, 26th (and 27th) vertebrae the conditions of the lumbar, sacral and coccygeal regions are commonly normal. But the developing ilium may become attached further anterior than usual, either directly to the costal process of the 24th vertebra or so far forward that a close ligamentous union is established with it. In such instances the 12th rib is usually either very rudimentary or absent and often the 29th vertebra is of the coccygeal type. In rare instances the thorax may at the same time advance a segment into the cervical region. On the other hand the developing ilium may take a position more posterior than usual, leaving the 25th vertebra either free to develop into the lumbar type or but incompletely united to the sacrum (Paterson, 93). When this occurs the 20th vertebra is very apt to have ribs developed in connection with it and the 30th vertebra usually becomes an integral part of the sacrum.

The coccygeal vertebrae, with the exception of the first, which is more directly than the others subjected to the differentiating influences of the developing limb, are relatively more rudimentary in the adult than in the embryo.

Rosenberg, 76, advanced the opinion that the ilium is attached more distally in the embryo than in the adult. I have recently, 1904, shown that this is not the case. On the contrary, as might have been inferred

from the distribution of the nerves to the posterior limb, the ilium is differentiated in a region anterior to the site of its permanent attachment and the differential activities which it stimulates in the sacral vertebrae are exerted first on the more anterior of these vertebrae.

The two limbs do not always call forth a similar response on each side of the body. Thus Paterson, 93, found asymmetry of the sacrum in 8.3% of the instances he studied.

Assuming the specific differentiation in the lumbar, sacral and coccygeal regions of the spinal column represents a response to stimuli arising in part from the developing limb, we may turn to a consideration of the differentiation thus brought about in each of these regions. Attention will here be directed chiefly to the more salient differences between development in the distal half of the vertebral column and that recently described for the thoracic region.

LUMBAR VERTEBRAE.

Rosenberg, 76, seems to have been the first to take up a detailed study of the early development of the lumbar vertebrae in man. He described the costal rudiment of these vertebrae and found in several embryos that this rudiment of the 20th vertebra had given rise to a cartilagenous 13th rib. A careful study of a large number of human embryos has led me, however, to the conclusion (1904) that a 13th rib is no more frequent in the embryo than in the adult and that in the series studied by Rosenberg it must have been unusually frequent. Holl, 82, found no 13th rib in the embryos which he examined. He also came to the conclusion that the transverse processes of the lumbar vertebrae do not represent ribs. Most investigators rightly disagree with him on this point.

The development of the external form of the lumbar vertebrae in a series of embryos belonging to the Mall collection is shown in Figs. 1-13, Plates I-V.

The bodies of the lumbar vertebrae during the earlier periods of differentiation are essentially like those of the thoracic vertebrae. In embryos over 12 mm. long, however, the former become progressively thinner, broader and longer than the latter. The intervertebral disks and the enveloping ligamentous tissue are similar in both regions. *In the thoracic region the canal of the chorda dorsalis lies nearer the ventral surface of the vertebral column than it does in the lumbo-sacral region. The marked alterations in the curvature of the spinal column which occur during embryonic development seem especially associated with changes in the intervertebral disks.

The neuro-costal processes of the lumbar vertebrae are also at first similar in form to those of the thoracic vertebrae. This is the case in Embryo II, length 7 mm., Fig. 1, Plate I; CLXIII, length 9 mm., Fig. 2, Plate II; and CIX, length 11 mm., Figs. 3 and 4, Plate II.

Marked differences in the costal processes are to be seen when chondrofication begins. Thus while the costal process of the 12th thoracic vertebra has early a separate center of chondrofication (Embryo CLXXV, length 13 mm., Fig. 14, Plate VI and Embryo CXLIV, length 14 mm., Fig. 5, Plate III), the processes of the lumbar vertebrae remain for a considerable period dense masses of mesenchyme (Embryo CLXXV, length 13 mm., Figs. 15 and 16, Plate VI, and Embryo CCXVI, length 17 mm., Figs. 18 and 19, Plate VI). Finally, however, they undergo chondrofication at the base (Embryo XXII, length 20 mm., Figs. 21 and 22). I have been unable to determine whether this chondrofication always takes place from a separate center, as it certainly often does, or sometimes represents merely an extension into the costal mesenchyme of cartilage from the transverse process. I incline to the former view.

Sometimes the costal element of the 1st lumbar vertebra may remain for a considerable period separate from the cartilage of the transverse process. This is true of the right side (left in the figure) of Embryo XLV, length 28 mm., Fig. 24. But usually at an early period the costal and transverse processes become intimately fused (Embryo XXII, length 20 mm., Fig. 21; XLV, length 28 mm., Fig. 24, right side of figure; and Fig. 25; Embryo LXXXIV, length 50 mm., Figs. 27 and 28). The "transverse" process of the adult lumbar vertebra represents in the main an ossification of a membranous, not cartilagenous, extension of the fused costal element (C. Pr., Fig. 28).

At first the neural processes of the lumbar vertebrae are essentially like those of the thoracic (Embryo CXLIV, length 14 mm., Fig. 5, Plate III). Union of the pedicles with the cartilage of the body takes place and the laminae extend out dorsally in a similar manner in each. It is in the transverse and articular processes that the chief characteristic differentiation takes place.

The lumbar transverse processes are broader and much shorter than the thoracic. At an early period, as mentioned above, they become intimately united to the costal processes. In the dense mesenchyme between the region of the transverse process and the costal element there is commonly developed no loose vascular area such as serves to separate the neck of the developing rib from the transverse process in the thoracic region. The occasional appearance of a foramen in a transverse process of a lumbar vertebra has led to the supposition that they may occur

regularly in the embryo (Szawlowski, 192, Dwight, 192). The differences between the transverse processes of the 12th thoracic and the first two lumbar vertebrae in several embryos are shown in Plates VI and VII, Figs. 14 to 28.

The articular processes in the lumbar, as in the thoracic, region are at first flat plates connected by membranous tissue,¹ Fig. 5, Plate III. But in the lumbar region the superior articular process develops faster than the inferior so that each superior process comes partly to enfold the inferior process of the vertebra next anterior. These conditions may be readily followed in Figs. 9, 12 and 13, Plates IV and V, and in Figs. 20-28, Plates VI and VII. The mammillary and accessory processes of the adult lumbar vertebrae probably represent an ossification of muscle tendons attached to the transverse and articular processes.

During the development of the vertebrae in embryos from 30 to 50 mm. in length alterations preliminary to ossification, similar to those in the thoracic, occur in the lumbar vertebrae. No actual ossification occurs in any of the centers in the latter in embryos less than 5 cm. long in Prof. Mall's collection. It begins in the bodies of the more anterior of the lumbar vertebrae in embryos between 5 and 7 cm. long, and in those 8 cm. long it has usually extended to the more distal. Meanwhile, ossification of the neural processes has extended from the thoracic into the lumbar region and soon may be seen throughout the latter. See Bade, 190.

SACRAL VERTEBRAE.

Although much has been written about the ossification of the sacral vertebrae comparatively little attention has been devoted to their early differentiation. Rosenberg, 196, contributed several important facts, although the general conclusions which he drew concerning the transformation of lumbar into sacral, and sacral into coccygeal vertebrae are unwarranted. Holl, 192, studied more especially the relation of the ilium to the sacrum in adults and embryos and added materially to the knowledge of the sacro-iliac articulation. Petersen, 193, has given an incomplete description of the sacrum in several early human embryos, and Hagen, 190, of one 17 mm. long.

Figs. 1 to 13, Plates I to V, show the general external form of the sacral vertebrae in embryos of the Mall collection. In Embryo II, length 7 mm., Fig. 1; CLXIII, length 9 mm., Fig. 2; and CIX, length 11 mm., Figs. 3 and 4, the sacral appear to resemble the lumbar in all essential particulars, although there is a progressive decrease in size from the mid-lumbar region distally. No line of demarcation can be drawn in these embryos between the sacral and lumbar vertebrae on the one side and the sacral and coccygeal on the other.

Soon after the stage shown in Figs. 3 and 4 the iliac blastema approaches more closely the vertebral column, usually in the region opposite the costal processes of the 25th and 26th vertebrae. These, then, are stimulated to more active growth and extend in their turn out toward the ilium. The costal processes of the 27th, 28th and 29th vertebrae are likewise stimulated into more active growth. Lateral to the ventral branches of the spinal nerves the tissue derived from the costal elements of these five vertebrae becomes fused into a continuous mass of condensed tissue (Fig. 37, Plate IX; and Figs. 5 and 6, Plate III). Against the anterior and better developed portion of this the iliac blastema comes to rest (Figs. 5 and 37). From the time of the fusion of the costal elements of the sacral vertebrae into a continuous lateral mass of tissue these vertebrae may be distinguished from the lumbar and coccygeal. Variation in the vertebrae entering into the sacrum occurs in the embryo as in the adult (Bardeen, 194).

At the period when the iliac blastema comes into contact with the costal mass of the sacrum centers of chondrofication have appeared in the bodies of the sacral vertebrae. The bodies, compared with the intervertebral disks, are progressively smaller from the first to the fifth (Embryo CXLIV, length 14 mm., Fig. 6). Otherwise they present no characteristics of special note. In older embryos this difference becomes less and less marked.

The neuro-costal processes present features of more specific interest. In Embryo CXLIV, length 14 mm., centers of chondrofication may be observed in the neural processes of the first two sacral vertebrae. They are not yet united to the bodies of the vertebrae and are simple in form. No cartilage has as yet appeared in the neural processes of the other vertebrae. In somewhat older embryos, CCXVI, length 17 mm., Fig. 38, and CLXXXVIII, length 17 mm., Fig. 39, the neural processes of all the sacral vertebrae have become chondrofied and distinct. Separate centers of chondrofication may be seen in each of the costal elements. At a slightly later stage, Embryo XXII, length 20 mm., Fig. 10, Plate IV, the extremities of the costal elements of the first three sacral vertebrae have fused with one another and have thus given rise to a cartilaginous auricular surface, and each has fused with the neural arch of the vertebra to which it belongs. The costal elements of the 4th and 5th sacral vertebrae are likewise fused to their corresponding neural arches and all of the sacral neural arches are fused to their respective vertebrae.

Cross-sections illustrate well the relations of the neural and costal processes to the vertebrae. Embryo CIX, length 11 mm., Fig. 29, Plate VIII, shows an early blastemal stage in which the sacral vertebrae resem-

ble the thoracic. Figs. 30, 31 and 32 represent cross-sections through the 1st, 2d and 3d sacral vertebræ of Embryo X, *length 20 mm.* The neural and costal processes in this embryo show cartilagenous centers, but these are fused neither with one another nor with the vertebral body. Embryo CCXXVI, *length 25 mm.*, Figs. 33 and 34, shows fusion of neural and costal processes with the body. The costal processes have by this period given rise to a continuous lateral cartilagenous mass, a portion of which is represented in Fig. 40. This shows an oblique section through the 3d and 4th sacral vertebræ of Embryo LXXXVI, *length 30 mm.* This cartilagenous lateral mass may likewise be seen in the sacra of Embryo CXLV, *length 33 mm.*, Figs. 11 and 12, and LXXXIV, *length 50 mm.*, Fig. 13.

From the primitive neural cartilages there develop pedicular, transverse, articular and laminar processes. The pedicular and transverse processes become intimately fused with the costal element as described above. In none of the embryos examined was there seen a separation of costal from transverse process marked by blood-vessels, such as one might perhaps expect to find because of the occasional appearance in the adult of transverse foramina in the lateral processes of the sacral vertebræ (Szawłowski, 192).

The articular processes in the older embryos retain a more primitive condition than do those of the lumbar and thoracic regions. Figs. 33, 34, 35 and 36 show cross-sections through the articulations of the neural processes.

The laminar processes of each side are still separated by a considerable interval in embryos of 50 mm., although at this period the lumbar region is nearly enclosed. Compare Figs. 28 and 36.

Changes in the cartilages preliminary to ossification occur both in the bodies and in the neural processes of the sacral vertebræ at a period quickly following their appearance in the lumbar region. Thus in Embryo LXXIX, *length 33 mm.*, changes of this nature may be followed as far as the 5th sacral vertebra. It is well known, however, that actual ossification in the more distal sacral vertebræ takes place considerably later than in the thoracico-lumbar region. The primary centers of ossification correspond with the centers of chondrofication except that there is a single center in place of two centers for each body, and one center instead of two for each neuro-costal processes of the two more distal sacral vertebræ. Posth, 97, has recently contributed a valuable paper on the subject of sacral ossification.

COCCYGEAL VERTEBRAE.

Since the valuable contributions of Fol, 85, who described 38 vertebrae in the early human embryo, considerable attention has been devoted to the development of the coccygeal vertebrae, especially from the point of view of the number of vertebrae in embryos. The literature on the subject has been summed up by Harrison, 01, and more recently by Unger and Brugsch, 03. The later stages in the development of the coccyx have been described by Steinbach, 89, who studied a large number of spinal columns of foetuses, infants and adults. In a recent paper, 1904, I have given a summary of the number of coccygeal vertebrae found in various embryos and of the number of haemal processes found in the embryos belonging to the collection of Prof. Mall.

Without attempting here to enter into a detailed account of the conditions found in these various embryos we shall pass at once to a consideration of the more characteristic features of coccygeal development. The differentiation of the coccygeal sclerotomes begins at about the end of the fourth week, Embryo II, *length 7 mm.*, Fig. 1, Plate I. As a rule at least six or seven membranous vertebrae are developed. The highest relative differentiation of the coccygeal vertebrae occurs in the fifth and sixth weeks. At this time dorsal processes connected by interdorsal membranes extend as far as the 4th and 5th vertebra, Figs. 3 and 4, Plate III, Fig. 41, Plate X. No distinct costal processes are, as a rule, developed on any except the first coccygeal vertebra, but at the height of development most of these vertebrae have distinct haemal processes, first described by Harrison, 01. These processes may also be seen on the more distal sacral vertebrae, Fig. 41, Plate X. They usually disappear before the embryo has reached a length of 20 mm., but the coccyx described by Szawlowski, 02, suggests that occasionally they are retained until adult life.

Usually the bodies of the first five coccygeal vertebrae become chondrified. The chondrofication of the more distal of these vertebrae is, as pointed out by Rosenberg, 76, often very irregular. There may be separate bilateral areas of cartilage or the two areas may be connected merely anterior to the chorda dorsalis. The bodies of successive vertebrae may be irregularly fused.

As a rule the neural processes of the first coccygeal vertebra alone become chondrosied and fused to the vertebral body. The others, as well as the connecting interdorsal membranes, disappear.

The cartilagenous coccygeal vertebrae are thus relatively less developed than the membranous. It is probable that the osseous are less developed than the cartilagenous. Thus although Steinbach, 89, has made a strong

plea for the presence of 5 vertebrae in the adult coccyx, the more commonly accepted number of four seems to be a truer estimate of the definite number of bones usually found present.

The bend of the coccyx which takes place during the third month is an interesting phenomenon. It seems to be associated with the development of pelvic structures.

SUMMARY.

In the earlier stages of development the lumbar, sacral and coccygeal vertebrae resemble the thoracic. The blastemal vertebrae arise each from the contiguous halves of two original segments of the axial mesenchyme. Each vertebra exhibits a body from which neural and costal processes arise. The neural processes are connected by "interdorsal" membranes.

As the blastemal vertebrae become converted into cartilage specific differentiation becomes more and more manifest. The cartilagenous vertebral bodies and the intervertebral disks are all formed in a similar manner and except for size manifest comparatively slight differences in form. The more distal coccygeal vertebrae are, however, irregular. But the chief specific differentiation is seen in the costal and neural processes.

In the blastemal neural processes of the thoracic vertebrae cartilagenous plates arise from which spring pedicular, transverse, articular and laminar processes.

In the lumbar vertebrae similar processes arise from the neural cartilages. The pedicular processes resemble the thoracic but are thicker; the transverse processes are shorter, much thicker at the base and remain bound up with the costal processes; the superior articular processes develop in such a way as to enfold the inferior; the laminar processes are broad, grow more directly backward than do the thoracic, and on meeting their fellows in the mid-dorsal line fuse and give rise to the typical lumbar spines. The mammillary and accessory processes are developed in connection with the dorsal musculature.

In the sacral vertebrae the neural cartilages give rise to very thick pedicular processes; to articular processes the most anterior of which develop like the lumbar, while the others long maintain embryonic characteristics; to transverse processes which in development are bound up with the costal processes; and to laminar processes which are very slow to develop and of which the last fail to extend far beyond the articular processes.

In the coccygeal vertebrae the neural processes of the first, and rarely

the second, alone give rise to cartilagenous plates. From these only pedicular and incomplete articular and transverse processes arise. The cornua of the adult coccyx represent fairly well the form of the early neural semi-arches. The transverse processes develop in close connection with the costal processes.

In the thoracic vertebræ cartilagenous ribs develop from separate centers in the blastemal costal processes.

In the lumbar vertebræ separate cartilagenous centers probably always arise in these processes, but they are developed later than those of the thoracic vertebræ and quickly become fused with the cartilage of the transverse processes. The transverse processes of the adult lumbar vertebræ represent at the base a fusion of embryonic cartilagenous costal and transverse processes, but in the blade an ossification of membranous costal processes.

In the sacral vertebræ separate cartilagenous costal centers are developed but they soon become fused at the base with the transverse processes of the neural plates. Laterally by fusion of their extremities the costal processes give rise to an auricular plate for articulation with the ilium.

In the coccygeal vertebræ the costal processes of the first become fused with the transverse processes and develop into the transverse processes of the adult coccyx. I have been unable definitely to determine whether a separate costal cartilage is developed in these processes or cartilage extends into them from the neural processes. The costal processes of the other coccygeal vertebræ have merely a very transitory blastemal existence.

For a brief period the more distal sacral and the coccygeal vertebræ have membranous haemal processes.

Centers of ossification correspond in general with centers of chondrofication, but, as in the case of the vertebral bodies and the more distal sacral neuro-costal processes, a single center of ossification may represent two centers of chondrofication.

B.

THE CURVES AND THE PROPORTIONATE REGIONAL LENGTHS OF THE SPINAL COLUMN DURING THE FIRST THREE MONTHS OF EMBRYONIC DEVELOPMENT.

In 1879 Achy contributed an important paper dealing with the length of the various regions of the spinal column at different ages, the height of the constituent vertebræ and the thickness of the intervertebral disks

in man. He showed that in young embryos the cervical region is relatively longer than the lumbar region but that as growth proceeds there is a constant proportional increase in length of the latter over the former. Taking the cervical region as 100, for instance, he found that in embryos below 10 mm. in length the lumbar region equals 69, while in the adult it is equivalent to 150. Thus, too, while from infancy to maturity the spinal column increases three and one-half times in length and the thoracic region at about the same relative ratio, the lumbar region increases four times in length and the cervical but three. Other investigators, including Ballantyne, 92, and Moser, 89, have in general confirmed these results. Of those who have studied the proportional length of the various regions in the adult Ravenel, 77, and Tenchini, 94, have made noteworthy contributions.

The post-natal lengthening of the lumbar region is associated with those changes in the lumbo-sacral curve which accompany the assumption of an erect posture during early childhood. Do similar alterations in relative regional length accompany the straightening of the spinal column which takes place during the first three months of embryonic development?

In Fig. 44, Plate X, I have represented by curved lines the vertebral columns of several embryos of this period and an adult column. The cervical, lumbar and coccygeal regions are represented by heavy, the thoracic and sacral regions by light lines. The 5th, 6th and 7th thoracic vertebrae are made to coincide in each instance.

The *anterior half* of the spinal column is considerably curved in Embryo II, *length 7 mm.* It gradually becomes straightened in successively older embryos until in Embryo CLXXXIV, *length 50 mm.*, it is nearly straight. The subsequent anterior convexity in the adult is associated with the assumption of an upright position of the head.

It is, however, in the *posterior half* of the spinal column that the chief alterations in spinal curvature are to be noted. In Embryo II, *length 7 mm.*, the ventral surface of the sacral region faces the mid-thoracic region; in Embryo CIX, *length 11 mm.*, the anterior end of the vertebral column; in Embryo CLXXXIV, *length 50 mm.*, almost directly ventrally; and in the adult, in a posterior direction.

The relative lengths of the various regions of the spinal column during the first three months of development may be gathered from the following table, which is based in part upon data obtained from embryos belonging to the Mall collection and in part upon those of the Born and His collections studied by Aeby.

TABLE A.

THE LENGTHS OF THE VARIOUS REGIONS OF THE SPINAL COLUMNS OF EMBRYOS OF THE SECOND AND THIRD MONTHS, AND THE PROPORTIONAL LENGTH OF THE THORACIC COMPARED WITH THE OTHER REGIONS.

Designation.	Collection.	Length in mm.	Regions of the spinal column.							
			Cervical.	Tho- racic.	Lumbar.	Sacral.	Coccygeal	Length in mm.	% of Thoracic region.	
			Length in mm.	% of Thoracic region.	Length in mm.	% of Thoracic region.	Length in mm.			
II.	Mall	7	2	60.6	3.3	1.2	36.4	.9	27.3	..
CCXXI.	"	13 ⁽¹⁾	2	62.5	3.2	1.3	46.1	1.25	39.1	1.0 31.2
1 (Aeby).	His.	10	2.95	76.6	3.85	1.75	45.5	1.95	50.6 ⁽²⁾	..
CIX.	Mall.	11	2.7	67.5	4	2	50.0	1.7	42.5	1.3 32.5
CXLIV.	"	14	2.3	62.2	3.7	1.7	46.0	1.34	36.2	1.1 29.8
XLIII.	"	16	2.9	61.9	4.34	1.9	48.7	1.55	35.7	1.41 32.5
2 (Aeby).	His.	10	3	65.2	4.6	2.25	48.9	1.88	40.9 ⁽²⁾	..
3 (Aeby).	"	16	3	64.5	4.65	2.25	48.2	2.65	57.0 ⁽²⁾	..
XXII.	Mall.	20	..	5	3	54.5	2.0	36.4	1	25
4 (Aeby).	His.	21.5	3.9	61.9	6.3	3.1	49.2	3.4	53.9 ⁽²⁾	..
CVIII.	Mall.	22	4.34	61.9	7.0	3.5	50.0	2.8	40	1.2 16.1
CXLV.	"	33	5.1	59.5	8.55	4	46.8	3.5	35.6	1.75 20
CCXXXVII.	"	30	5.4	60.0	9.0	4	44.4	3	33.3	1.38 15.3
5 (Aeby).	Born.	..	6.0	54.5	11	5.2	47.3
XCV.	Mall.	46	8.25	59.0	14	7.25	51.8	5.5	39.3	3.75 26.8
CLXXXIV.	"	50	8	61.1	13	6.35	48.8	4.65	35.8	2.65 20.4
6 (Aeby).	Born.	..	10	58.8	17	8.5	50.0	9.5	55.8 ⁽²⁾	..

1. This is the measurement recorded before the embryo was sectioned. The embryo was cut sagittally. The length of the sections in the median line is 7.5 mm. The general development corresponds with that of an embryo of this length.

2. These figures represent the length of the pelvic portion of the spinal column.

This table discloses considerable individual variation. The length of the cervical region is about 60% of that of the thoracic. In Embryos CIX, 1 and 2, this ratio is much exceeded. The measurements for CIX are calculated from obliquely transverse sections and hence are subject to some error. The data concerning the measurements for 1 and 2 are not given by Aeby.

The lumbar region, at first less than 40% in length of the thoracic, in most embryos approximates 50%. The length of the sacral region varies from 33 to 42.5% of the thoracic. The coccygeal region, with a nearly constantly diminishing comparative length, shows marked variations.

There is no good evidence that the straightening of the spinal column is accompanied by a marked increase in relative length of the lumbar region after the early stages in Embryos II and CIX.

A comparison of the spinal columns of embryos of the second and third months with those of older embryos and of children shows that it is during the latter half of foetal life and early childhood that the chief relative lengthening of the lumbar region takes place.

According to Aeby the average length of the cervical, thoracic and lumbar regions in the new-born is respectively 45.1, 83.9 and 47.5 mm. This makes the length of the cervical region 53.5% and that of the lumbar region 56% of that of the thoracic. Corresponding figures from Ballantyne, 92, for full-term foetuses are: cervical, 33.6 mm. (42.8%); thoracic, 78.4 mm. (100%); lumbar, 42.8 mm. (54.3%); and sacro-coccygeal, 39.8 mm. (50.8%). Thus Ballantyne finds a greater proportional reduction of the cervical region.

The conditions in the adult, as given by various investigators, are as follows:

TABLE B.

PROPORTIONAL LENGTHS OF THE VARIOUS REGIONS OF ADULT SPINAL COLUMNS.

Investigator and date.	Sex and Size.	Average length of regions in mm.				Ratio of other regions to the thoracic as 100.		
		Cervical.	Thoracic.	Lumbar.	Sacro- coccygeal.	Cervical.	Lumbar.	Sacro- coccygeal.
Ravenel, 1877.	Male.	133	280	182	..	47.5	65.0'	..
	Female.	120	260	178	..	46.1	68.5	..
Aeby, 1879.	Male.	129.9	273.4	184.1	..	47.5	67.3	..
	Female.	122.9	265.8	190.3	..	46.2	71.6	..
Tenchini.	Short.	98	222	125	151	44.1	56.3	68
	Medium.	100	240	137	158	41.7	57.1	65.8
	Tall.	104	240	134	168	43.3	55.8	61
Dwight, 1894.	Male.	133	287	19.9	..	46.3	69.3	..
	Female.	121	265	18.7	..	45.7	70.6	..

The chief point of interest in this table is the difference between the results found by the German and American investigators and those of the Italian. Apparently the Italians have proportionately shorter cervical and lumbar regions than the Americans and Germans, but it is possible that different ways of measuring were used. It is a subject worthy of further investigation.

Both Tenchini and Aneel and Seneert, 102, have treated of variations in measured length of individual vertebrae associated with numerical vertebral variation.

C.

THE DEVELOPMENT OF THE SKELETON OF THE POSTERIOR LIMB.

One of the most studied subjects in morphology has been the development of the vertebrate limbs. Fortunately critical summaries of its immense literature have recently been given by several keen investigators, among whom may be mentioned Wiedersheim, 92, Mollier, 93, 95, 97, Gegenbaur, 98, 01, Klaatsch, 00, Rabl, 01, Fürbringer, 02, Ruge, 02, and Braus, 04. Therefore no attempt will here be made to review previous work except in so far as it deals directly with the development of the human limb.

During the third week of embryonic life the limb-buds become filled with a vascular mesenchyme (Bardeen and Lewis, 01, p. 17, Figs. 18 and 19). The source of this tissue is uncertain. In part it may come from the primitive body-segments, but it seems probable that in the main it comes from the parietal layer of the unsegmented mesoderm.

During the fourth week the mesenchyme increases in amount and the limb-bud begins to protrude further from the body-wall. Observed structural differentiation does not, however, begin until the early part of the fifth week, at the time when the lumbo-sacral spinal nerves are beginning to form a plexus. At this period the tissue at the center of the base of the limb becomes greatly condensed, Embryo CLXIII, *length 9 mm.*, Fig. 45, Plate XI. The boundaries of the mass are not perfectly definite, but a wax-plate reconstruction based upon drawings made as definite as possible gives rise to the structure shown in Fig. 2, Plate II. The relations of this tissue mass to other structures are shown in Plate II, Fig. B, and Plate III, Fig. C, of the article by Bardeen and Lewis, 01. The condensation represents the acetabulum and the proximal end of the femur. This is indicated by its relations to the nerve plexus.

Once begun skeletal differentiation proceeds rapidly. In Embryo CIX, *length 11 mm.*, Figs. 3 and 4, it may be seen that from the original center of skeletal formation the condensation of tissue has extended both distally and proximally, but much more rapidly in the former than in the latter direction. Distally the sclero-blastema shows femur, tibia, fibula, and a foot-plate; proximally, an iliac, a public and an ischial process. A series of sections through the skeletal mass (Figs. 46 to 52) shows that in the femur, tibia and fibula chondrofication has begun. At the centers of the blastema of the ilium, ischium and pubis a still earlier stage of chondrofication has made its appearance. The leg of this embryo, therefore, represents a stage of transition from the blastemal to the chondrogenous stage of development. Fig. 55, Plate XII, shows a longitudinal

section through the leg of Embryo CLXXV, *length 13 mm.* The development is slightly more advanced than in Embryo CIX.

The further general development of the skeleton of the limb may be followed in Figs. 8 to 13. For the sake of convenience the development of the several parts of the skeleton will be taken up in turn as follows: (a) pelvis; (b) femur, hip-joint, tibia and fibula, and knee-joint; (c) ankle and foot.

A. PELVIS.

Petersen, 93, has given a good account of the early development of the human pelvis. His work was based upon embryos belonging to the His collection. In embryos Ru, *length 9.1 mm.*, Ko, *length 10.2 mm.*, and N, *length 9.1 mm.*, the formation of the lumbo-sacral plexus has begun and there is a condensation at the center of the limb-bud. The conditions here resemble those in Embryo CLXIII, *length 9 mm.*, Fig. 2, described above. Petersen believed that the condensation of tissue in embryos Ru and Ko represents the germinal area for the muscles and skeleton of the lower extremity while that of N shows a further differentiation of the diaphysis of the femur. The last ends anteriorly in a small undifferentiated cell-mass but there is nothing further to indicate the future pelvis. Yet, as I have mentioned above, the relations of this cell-mass to the nerves arising from the plexus indicates that it is the fundament of the future pelvis. The nerves pass about it as they do later about the acetabulum. In Embryo S₁, *length 12.6 mm.*, Petersen found what he considers the first traces of a definite pelvic fundament. This embryo is evidently of about the same stage of development as CIX, *length 11 mm.*, Figs. 3 and 4 and 46 to 53. But CIX is slightly more advanced and shows early stages of chondrofication not seen in S₁. The pelvis of S₁ has a slightly more anterior position and the iliac blastema extends rather toward the 24th than the 25th vertebra (Fig. 1, Plate I, of Petersen's article).

The pelvic scleroblastema of embryos of the stage found in CIX undergoes a rapid development. Its iliac portion extends in a dorsal direction toward the vertebræ which are to give it support. The costal processes of the latter at the same time become fused into an auricular plate. With this the iliac scleroblastema comes into close approximation, Figs. 5 and 6, Plate III, although for some time separated by a narrow band of tissue staining less densely than the blastemal, Fig. 37.

Anteriorly the iliac blastema extends toward the abdominal musculature, to which it finally serves to give attachment.

While the blastemal ilium is thus becoming differentiated the pubic

and ischial processes of the pelvic blastema extend rapidly forward and ventral to the obturator nerve they become joined by a condensation of the tissue lying between them. Thus the obturator foramen of the blastemal pelvis is completed, Figs. 5 and 6. Between the crest of the ilium and the ventral extremity of the pubis dense tissue is formed to give attachment to the oblique abdominal musculature. This represents the embryonic Poupart's ligament and completes a femoral canal, Figs. 5 and 6.

While the blastemal pelvis is being differentiated the formation of cartilage in the ilium, ischium and pubis extends rapidly from the centers indicated in Embryo CIX. In CXLIV, *length* 14 mm., Figs. 5, 6 and 54, the three cartilages are distinct.

The iliac cartilage is a somewhat flattened rod with anterior and posterior surfaces, Fig. 38. The anterior surface of the iliac cartilage at first faces slightly laterally as well as anteriorly. Lubsen, 193, in an interesting paper has shown the importance of this from the standpoint of mammalian phylogeny. He considers a flat plate with median and lateral surfaces to be the probable primitive form of ilium from which the triangular form, on which Flower, 70, laid stress, is derived by a lateral projection serving to divide the lateral surface into an anterior iliac and a posterior gluteal portion. In man and some other mammals the anterior iliac surface, according to Lubsen, comes to be turned medially by a great extension of the lateral projection and a secondary union of the abdominal musculature to this. In man, however, the primitive iliac cartilage is a rounded plate of which the long axis of the cross-section lies nearly at right angles to the median plane of the embryo. On the whole it suggests the prism described by Flower.

The pubic and ischial cartilages when first formed are mere rounded masses of tissue lying in the center of their respective blastemal processes. The acetabulum at this time is composed mainly of blastemal tissue but the iliac and ischial cartilages form a part of its floor, Embryo CXLIV, *length* 14 mm., Figs. 5 and 6, Plate III. The pelvis of CR, *length* 13.6 mm., described by Petersen and pictured in Fig. 2, Plate I, of his article, is of a stage of development similar to that of CXLIV. The cartilages are slightly more advanced in development. The iliac cartilage is broader in an antero-posterior direction and extends to the sacrum. He observed no dense tissue completing the obturator foramen but this tissue is quite plain in the corresponding embryos of the Mall collection, and it is described by Petersen for embryos Wi, *length* 15.5 mm., and Ob, *length* 15 mm., which are slightly more advanced than CR.

While the human embryo is growing from 15 to 20 mm. in length, there occurs a rapid development of the pelvic cartilages. About the head of the femur each gives rise to a plate-like process. The fusion of these processes produces a shallow acetabulum, Figs. 9 and 10, Plate IV. Those from the ilium and ischium are larger than that of the pubis and fuse with one another before the pubic cartilage fuses with them. The proportional areas of the acetabulum to which each pelvic cartilage contributes seem to be essentially the same as those later furnished by the corresponding pelvic bones, $\frac{2}{5}$ + ischium, $\frac{2}{5}$ — ilium, $\frac{1}{5}$ pubis. While growing about the hip-joint so as to complete the acetabulum each of the pelvic cartilages has a centrifugal growth within the blastemal pelvis. It is convenient to consider each cartilage in turn.

The iliac cartilage of the stage represented in Embryo CXLIV, Figs. 5 and 6, represents essentially that portion of the ilium which borders the entrance to the true pelvis and which has hence been called the pelvic portion of the ilium. From this cartilage extends dorsally into the sacral area of the blastemal pelvis, Figs. 9 and 10, and there gives rise to the sacral portion of the cartilagenous ilium. At the same time cartilage extends into the blastema which passes anteriorly to give attachment to the abdominal musculature and from this arises the abdominal portion of the cartilagenous ilium. A slight extension of cartilage into the anlage of Ponpart's ligament forms the anterior superior spine, but the blastemal covering of the femoral canal is not converted into cartilage as is the similar covering of the obturator.

While the cartilagenous ilium is being developed the ischial and pubic cartilages extend ventrally into their corresponding blastema. The ischial cartilage rapidly increases in thickness and at the same time gives rise to two processes. One of these, the ischial spine, extends toward the sacrum. This seems to indicate a commencing enclosure by cartilage of the ilio-sciatic notch. The other projects toward the developing hamstring muscles and gives rise to the ischial tuberosity.

The pubis broadens as it extends forwards and beyond the obturator foramen sends down a process which fuses with a longer one extending up from the ischium.

The various stages in the formation of a cartilagenous innominate bone have been followed in detail by Petersen in a series of six embryos from 17.5 to 22 mm. in length. In general what he describes coincides well with the appearances presented in a somewhat more extensive corresponding set of embryos belonging to the Mall collection. The distal position of the ilium, represented in Fig. 12, Plate VII, of Petersen's article, is to be looked upon as an individual variation and not as a

regular step in the process of attachment of ilium to spinal column. This I have previously pointed out (1904). Hagen, 90, has given an account of the pelvis of an embryo of 17.5 mm., which corresponds with the description given above.

During the further development of the cartilaginous pelvis the ventral extremities of its two halves, at first widely separated, in embryos of 20 mm. in close proximity, are finally united by a symphysis when the embryo reaches a length of 25 mm. In an embryo of this length, CCXXVI, the blastemal tissue of each half is fused in the median line but the cartilages are separated by $\frac{1}{2}$ mm., although the width of the pelvis at the rim is only 3 mm. In this embryo the obturator foramen is completely enclosed by cartilage. In embryos of 30 mm. the pubic cartilages are closely approximated in front.

Petersen reconstructed the pelvis of an embryo of 29 mm., Lo_1 , and has given an extensive description of it. In essential features it corresponds with the pelvis of Embryo CXLV, *length 33 mm.*, Figs. 11 and 12. The sacrum of this latter embryo is, however, composed of the 25th to 29th vertebrae, while Petersen found the 30th vertebra of Lo_1 belonging to the sacrum. This variation is common in the adult.

Of the characteristic features common to Lo_1 and CXLV may be mentioned the relatively great development of the sacral portion of the ilium, with a large posterior-superior spine, the relatively slight development of the abdominal portion, and the comparatively large part of the pelvic entrance which is bounded by the sacrum. In the adult, according to Engel; the sacrum bounds 26.2% of this. For the new-born female the following figures are given by Fehling: sacrum, 28.9%; ilea, 29.2%; pubes, 42.8%; for the new-born male, 30.4%, 26.9% and 42.2%. For Lo_1 , the percentages are: 37.0%, 31.7% and 31.3%; for CXLV, 34%, 33% and 33%.

In Lo_1 the rim of the acetabulum is deepened by dense blastemal tissue. In CXLV this has been in part converted into cartilage by extension of processes from the ilium, ischium and pubis. The processes of the ischium and pubis are fused with that of the ilium but not with one another, so that the cotyloid foramen is well marked.

In both embryos the iliac blades bend more sharply than in the new-born and resemble in this respect the adult. The ischial spines are relatively more developed and project more into the pelvis than do those of the adult.

The pelvis of Lo_1 is that of a female; the pelvis of CXLV that of a male. It is of some interest to determine whether or not sexual differentiation is apparent. Fehling, 76, showed that during foetal life differ-

ences of this nature, though by no means marked, are none the less to be made out. His conclusions have been confirmed by Veit, 89, Romiti, 92, Konikow, 94, Thomson, 99, and Merkel, 02. Petersen has made most careful comparisons between the measurements of the pelvis he reconstructed and the structural data furnished by Fehling. The variations due to sex are, however, so slight that they are likely to be obscured in wax reconstructions of early embryos. Allowance must be made for errors of technique and for the difficulty of determining corresponding points between which measurements are to be taken. Thus, for instance, the proportional widths of the entrance to the true pelvis, the pelvic cavity and the pelvic exit I find to be in CXLV as 100:75:54, while Petersen makes them for Lo₁ as 100:74.7:46.1. According to theory the width of the exit should be proportionately less in the male than in the female pelvis. For foetuses of 30 to 34 cm. Fehling gives for females 100:88:70; for males 100:87:60; for new-born females 100:84:76; for new-born males 100:82:65; for adults 100:92:81. Without the possibility of a direct comparison of the two reconstructed pelvises it is therefore scarcely possible to determine accurately to what extent they may show sexual differences. A characteristic on which Merkel, 03, lays especial stress, the more posterior position of the greatest width at the pelvic entrance in the male, does, I think, exist in CXLV in comparison with Lo₁.

These two embryos show that at the beginning of the third month of development the cartilagenous pelvis is well formed. At this time, also ossification begins in the ilium. Preliminary changes in the cartilage may be seen in embryos of 25 mm. in an area corresponding to that in which chondrofication commenced. These changes are further advanced in CXLV, but in this embryo neither deposit of calcium salts nor true ossification has commenced in the ilium although ossification is under way in the clavicle, inferior and superior maxillæ, occipital, humerus, radius, ulna, femur, tibia and fibula. Another embryo of the same length, 33 mm., LXXIX, does, however, show a well-marked area of ossification, Fig. 58. In the endochondral region calcium salts are deposited while on each side of this perichondral ossification takes place. In an older embryo, LXXXIV, length 50 mm., this latter process shows well, Fig. 36. It is known that ossification of the ischium and pubis takes place considerably later, that of the ischium beginning in the 4th month and that of the pubis in the 6th to 7th (Bade, 00).

Aside from the ossification of the ilium nothing especially noteworthy seems to take place in the development of the pelvis during the period

when the embryo is growing from 30 to 50 mm. in length. Fig. 13 shows the form of the pelvis in an embryo of the latter size.

The relations of the pelvis to the sacrum during the second and third months of life deserve some attention. I have endeavored to illustrate them in Fig. 44. The curves of the spinal columns of several embryos and an adult are there shown. A point "a" represents the place where a line joining the centers of the two acetabula would cut the median plane of the embryo. From this point dotted lines are drawn to each extremity of the sacral region and one is projected perpendicularly to a line joining these two extremities. A fourth line from point "a" indicates the direction of the long axis of the femur.

In Embryo II, *length 7 mm.*, the leg skeleton is not differentiated. "a" represents there the approximate position where the pelvic blastema will first become marked, as in Embryo CLXIII, *length 9 mm.* The perpendicular falls on the body of the 1st sacral vertebra and points toward the mid-thoracic region.

In Embryo CIX, *length 11 mm.*, the perpendicular falls on the 1st sacral vertebra; in CXLIV, *length 14 mm.*, about at the junction of the 2d and 3d; in CVIII, *length 22 mm.*, on the 3d; in CXLV, *length 33 mm.*, at the junction of the 2d and 3d; in CLXXXIV, *length 50 mm.*, on the anterior portion of the 3d.

At birth, judging from the figure of Fehling, the perpendicular would strike at about the junction of the 2d and 3d. In the adult the area where it strikes shows much individual variation, but in most of the specimens which I have examined it strikes on the 2d sacral vertebra not far from the junction of this with the 3d. In some specimens it strikes the 3d. The material at my disposal has been chiefly dried specimens from the dissecting room and has been subjected to some warping.

Fig. 44 shows that when first differentiated the pelvis occupies a position anterior to that which it takes when it becomes attached to the vertebral column, but that after this attachment the position of the central area of the acetabulum is altered but slightly with respect to the sacral region of the vertebral column. At the beginning and at the end of the period under consideration it probably occupies a position slightly more anterior than that which it takes during the latter part of the second and first part of the third month of development. The chief alteration of the position of the pelvis with respect to the long axis of the body is due to change in the position of the sacrum in relation to the rest of the spinal column.

Merkel, 192, has contributed an important paper on the growth of the

pelvis and refers to the previous literature on that subject. He shows that the sacrum and the innominate bones exhibit a certain independence in rate of growth.

B. FEMUR AND HIP-JOINT, TIBIA, FIBULA, AND KNEE-JOINT.

The rapid development of the blastemal skeleton of the lower limb has been briefly described above. Soon after the fundament of the femur makes its appearance condensation of tissue marks out the anlage of the tibia and fibula and the skeleton of the foot. This last seems to be at first a somewhat irregular continuous sheet of tissue. It is not clear whether or not the anlage of the tibia and fibula also begins as a continuous tissue sheet which becomes divided, by ingrowth of blood-vessels, into tibial and fibular portions. The incomplete development of the interosseous fissure in Embryo CIX, *length 11 mm.*, Figs. 3 and 4, suggests this. The blastemal anlagen of the tibia and fibula are here very incompletely separated.

In Embryo CIX the femoral blastema is continuous at one end with that of the pelvis, at the other with that of the tibia and fibula and that of the last two with the foot-plate.

Within the blastema of the femur, tibia and fibula chondrofication begins as soon as the outlines of the blastemal skeleton are fairly complete (Figs. 3 and 4). The embryonic cartilage appears slightly knee-wards from the center of the shaft of each bone and then extends toward the ends. In CIX, Figs. 3 and 4, and 46 to 53, the cartilage of the femur consists of a bar largest at the knee whence it tapers off toward the hip. The cartilages of the lower leg lie nearly in a common plane. That of the tibia is larger than that of the fibula and toward the knee it broadens out considerably. At this stage the joints consist of a solid mass of mesenchyme, Fig. 55. The tissue uniting the femur and tibia has something the appearance of precartilage.

The further development of the thigh and leg may be conveniently studied by taking up at first the development of the femur and hip-joint, and then that of the tibia, fibula and knee-joint.

The cartilagenous femur expands rapidly at the expense of the surrounding blastemal perichondrium and at the same time acquires adult characteristics.

In an embryo of 14 mm., CXLIV, Figs. 5 and 6, the shaft of the femur extends almost directly into the hip-joint. Here there is a simple rounded head, distal and dorsal to which a slight projection marks the beginning of the great trochanter. There is nothing corresponding to a true

"neck." Similar conditions have been pictured by Hagen, 60, for the His embryo So, *length 17 mm.*

In Embryo XXII, *length 20 mm.*, Figs. 9 and 10, the head of the femur is proportionately larger and between it and the great trochanter the cartilage has developed in such a way as to give rise to a short neck. Blastemal extensions serve to give attachment to the musculature of the hip and indicate the lesser trochanter and the intertrochanteric ridge. In Embryo CXLV, *length 33 mm.*, Figs. 11 and 12, the cartilage has extended into these projections and the main characteristics which distinguish the proximal end of the femur have become established. Even at this stage, however, the neck is proportionately very short and thick. In an embryo of 50 mm., LXXXIV, Fig. 13, the neck is relatively more slender and the head of the femur has become more rounded.

The hip-joint is represented at first by a dense mass of scleroblastema, Fig. 55. The development of the acetabulum by ingrowth and fusion of processes from the iliac, ischial and pubic cartilages has already been described. The cartilagenous joint-cavity is at first quite shallow, Fig. 56. But extension of cartilage into the blastemal tissue which passes from the pelvis over the head of the femur serves greatly to deepen it on all sides except in the region of the cotyloid notch.

The joint-cavity is at first completely filled with a dense blastemal tissue, Fig. 56. While the embryo is growing from 20 to 30 mm. in length cavity formation begins in the tissue lying between the cartilagenous floor of the acetabulum and the head of the femur. The first stage in the process is marked by a condensation of the capsular tissue immediately bordering upon the joint and of the perichondral tissue which at this stage covers the cartilages on their articular surfaces as well as elsewhere. In the region of the ligamentum teres a fibrous band is likewise differentiated from the blastema of the joint. The rest of the tissue becomes looser in texture and ultimately is absorbed, Fig. 57. Henke and Reyher, 74, gave a good account of the development of the hip-joint. Moser has discussed the ligamentum teres.

The shaft of the femur at the stages of Embryos CIX and CXLIV, Figs. 3, 4, 5 and 6 is proportionately very short and thick. For a time it then grows so rapidly that it may become distorted and bent from the resistance offered at each end. But soon adjustment takes place between the skeletal and the neighboring parts and the femur becomes straight and slender. Yet in Embryo CXLV, *length 33 mm.*, Figs. 11 and 12, it is relatively thicker than in the adult.

The *linea aspera* is marked during the early development of the femur by a thickening of the perichondrium in the region where the various

muscle tendons and fascia are inserted. But in embryos up to 50 mm. in length there is no extension of cartilage into this area. Since by this period the shaft is ossified it is evident that no cartilagenous linea aspera is formed.

Ossification begins at an early period kneewards from the center of the shaft. Endochondral calcification begins here in embryos about, or slightly less than 20 mm. in length. Perichondral ossification usually begins in embryos about 25 mm. long, although in Embryos LXXXVI and LXCV, *length 30 mm.*, the clavicle alone shows actual bone formation. Ossification of the femur takes place at about the same time as that of the humerus, radius and ulna, and very slightly, if at all, precedes that of the tibia. Ossification of the clavicle and the superior and inferior maxillary bones seems always to begin a little earlier, that of the scapula, ilium, occipital, and ribs, slightly later.

The distal extremity of the femur is large at an early period of differentiation, Embryo CIX, Figs. 3 and 4. In Embryo CXLIV, *length 14 mm.*, Figs. 5 and 6, it has expanded laterally and each lateral process has extended dorsally so that fairly well-marked condyles are apparent. These are better formed in Embryo XXII, *length 20 mm.*, Figs. 9 and 10. In CXLV, *length 33 mm.*, Figs. 11 and 12, the form of the distal extremity of the femur resembles the adult.

The tibia and fibula at first lie nearly in the same plane, Embryo CIX, *length 11 mm.*, Figs. 3 and 4. As the head of the tibia enlarges toward the knee-joint it comes to lie dorsal to the proximal extremity of the fibula. This may be seen in Embryo CXLIV, Figs. 5 and 6, and more marked in Embryo XVII, *length 18 mm.*, Fig. 59; XXII, *length 20 mm.*, Figs. 9 and 10; and CXLV, *length 33 mm.*, Figs. 11 and 12. In the last embryo the relations of the head of the fibula to that of the tibia are nearly like the adult.

In Embryo CIX, Figs. 3 and 4, the fibula points toward the lateral condyle of the femur and the tibia toward the median, but the long axis of the femur much more nearly meets that of the tibia than that of the fibula. As the head of the tibia enlarges the anterior extremity of the long axis of the bone is carried toward the center of the distal end of the femur while the head of the fibula is pushed toward the side, Figs. 5, 6, 59, 9, 10, so that the long axis of the fibula comes to point lateral to the extremity of the femur. The head of the fibula is held in place by ligaments developed from the skeletal blastema.

The development of the *knee-joint* in man has been studied by a number of competent observers. Bernays, in 1878, gave a good review of the previous work of von Baer, Bruch, Henke and Reyher, and an

accurate description of the processes which take place. Of the more recent articles that of Kazzander, 94, deserves special mention.

Until the embryo reaches a length of about 17 mm. the knee-joint is marked by a dense mass of tissue, Fig. 59. The medullary tissue at the knee, like that at the hip and other joints, is less dense than the surrounding cortical substance, so that when the cartilages of the femur, tibia and fibula are first differentiated they seem to be connected by a tissue which, in some respects, resembles the prochondrium of which they are composed, Fig. 55. But as the cartilages become more definite the apparent continuity disappears. As the musculature becomes differentiated a dense tendon for the quadriceps is formed in front of the knee-joint. This is shown well in Fig. 56. In it the patella becomes differentiated.

In embryos of about 20 mm. the tissue immediately surrounding the cartilages becomes greatly condensed into a definite perichondrium. The peripheral blastemal tissue at the joints becomes transformed into a capsular ligament strengthened in front by the tendon of the quadriceps. Within the joint most of the tissue begins to show signs of becoming less dense, Fig. 56, but the semi-lunar disks and the crucial ligaments, like the ligaments of the capsule are differentiated directly from the blastema, Figs. 61 to 65. A knee-joint cavity first appears in embryos about 30 mm. long.

The shafts of the tibia and fibula are incompletely separated in the blastemal stage. The cartilages which arise in the scleroblastema are, on the other hand, separated by a distinct interval, Fig. 50, and as the blastemal elements give way to cartilage the interosseous space becomes larger. This is shown in Figs. 3, 4, 5, 6, 9, 10, 11, 12 and 59. At first short and thick the shafts gradually become more slender in proportion to their length. The fibula at all times smaller, becomes increasingly more slender in comparison with the tibia. In embryos of 30 mm., Figs. 11 and 12, both bones, and especially the fibula, are relatively thick compared with the adult bones.

During a period of rapid development, in embryos of 15 to 20 mm., the tibia and fibula, like the femur, may extend so rapidly in length as to become temporarily distorted by resistance at the ends. This is often especially marked in hardened specimens. Holl, 91, Schomburg, 100, and others have called attention to this distortion.

Ossification begins in the tibia at about the same time that it does in the femur and a little earlier than it does in the fibula. It is usually under way in embryos 25 mm. long. In older embryos it is generally well marked, Figs 11 and 12, Plate V. It begins in both bones knee-wards from the center of the shaft and from here spreads toward the

ends of the bones (Fig. 13, Plate V). The development of the distal extremities of the tibia and fibula may best be taken up in connection with the development of the foot.

C. ANKLE AND FOOT.

Of the papers dealing with the early development of the skeleton of the human foot the more important are those of Henke and Reyher, 74, Leboucq, 82, v. Bardeleben, 83, 85, Lazarus, 96, and Schomburg, 100.

Since the work of Schomburg is the most recent of these and is based on a considerable number of well-prepared embryos, I shall discuss his results somewhat at length in connection with the results which I have obtained. He recognizes four periods in the development of skeletal structures, a mesenchymal, a prochondral, a cartilagenous and an osseous. For the sake of ready comparison I shall take up each of these periods in turn. The fourth period falls within the scope of this paper only in so far as it overlaps the third.

Mesenchymal (blastemal) period.—This commences during the fifth week of embryonic development. The free extremity of the limb-bud becomes flattened and differentiated into the anlage of the foot and its axial blastema becomes differentiated into a foot-plate, from which later the bones of the foot are derived. Schomburg states that the axial blastema becomes distinct at the end of the fourth week. In Embryos CCXLI, *length 6 mm.*; II, *length 7 mm.*; CLXIII, *length 9 mm.*; and CCXXI, *length 13 mm.*,¹ I find no distinct signs of a foot-plate. In each of the following embryos I find a foot-plate which has not distinctly undergone further differentiation: CIX, *length 11 mm.*; CLXXV, *length 13 mm.*; and CVI, *length 17 mm.* The last is a somewhat pathological specimen. In Fig. 3 a reconstruction of the foot-plate of CIX is shown, in Figs. 51 and 52 transverse sections through this are represented, in Fig. 66 is pictured a longitudinal section through the foot-plate of CLXXV.

Toward the end of the fifth week, in embryos usually 14 to 16 mm. long, the first differentiation of definite bones is manifested by a condensation of tissue in specific areas. Within these areas of condensed tissue precartilage soon makes its appearance. Schomburg says that the first metatarsal is differentiated distinctly later than the other metatarsals. This I find to be the case in none of Prof. Mall's embryos. I do, however, agree with Schomburg that the metatarsal bones become well differentiated before the tarsals. When the metatarsals and phalanges

¹ See note 1, Table A, p. 277.

become differentiated the portions of the foot-plate between them serve for a short time to form a thick web, Fig. 67.

Prochondrium period.—Schomburg gives a detailed account of the early differentiation of the anlagen of the bones of the foot and illustrates his belief as to their nature by several diagrams. Unfortunately he does not picture the wax-plate reconstructions which he reports having made of a number of early embryos. In Prof. Mall's embryos I find no evidence of the archipterygium-like conditions which Schomburg describes. While it may be true that the somewhat slow development of cartilage in the tarsus is owing to the great alterations from primitive conditions which the human foot has undergone during its phylogeny, and to a certain extent has to repeat during its ontogeny, still the development of the bones of the foot is far more direct than Schomburg's diagrams indicate. In the embryos studied I also fail to find the rudimentary tarsal bones described by v. Bardeleben, 83, 85. I have examined six embryos between 15 and 20 mm. long without finding a trace of either the os intermedium tarsi or the triangularis tarsi. In only one instance have I found the I cuneiform distinctly portioned out into dorsal and plantar divisions by a lateral fissure. Study of adult variation statistically, as so admirably carried out by Pfitzner, 96, for the foot, coupled with comparative anatomy, in this, as in so many other fields, throws more light on a possible phylogeny than is gained from ontological investigation.

Embryo CXLIV, *length 14 mm.*, is, of those I have studied, the youngest showing definitely tarsal and metatarsal elements. The general form of the skeleton is shown in Figs. 5 and 6. The differentiation of the tarsal elements is difficult to make out, that of the metatarsals is clear. Webs between the latter still persist, Fig. 67. Webbed digits are sometimes found in the adult (Robertson).

It is to be noted that the elementary condition of the foot of CXLIV corresponds with none of the diagrams given by Schomburg. On the whole the cartilagenous anlagen have a position much more nearly resembling the adult. Embryo XLIII, *length 16 mm.*, exhibits pedal characteristics almost identical with those of CXLIV.

It may here be mentioned that in none of the embryos I have studied is the fibula so long as the tibia. Schomburg states that at first it is longer.

The metatarsals when first formed are spread wide apart and gradually become approximated. The diagrams of Schomburg indicate a different condition.

Cartilagenous period.—This Schomburg distinguishes from the preceding by the fact that cartilage cells at the centers of the areas of chondrofication show definite cell boundaries and become larger than the surrounding prochondral cells. These changes take place in the various skeletal anlagen in the order in which the anlagen were originally formed. With the active production of cartilage cells the broad surrounding zone of mesenchyme gives way to a narrower, denser perichondrium. At the same time the form of the skeleton becomes more definite, so that, as Schomburg says, the cartilages of the foot of an embryo at the middle of the third month give a good picture of the adult bones of the foot. The articular surfaces acquire more or less their definite form. I quite agree with Schomburg, in opposition to Henke and Reyher, that the joints of the foot, like the other joints of the body, are laid down at the start in their definite form and are not moulded into shape by use.

The skeleton of the foot at the time when the cartilage cells at the centers in most of the bones are beginning to be distinctly outlined, has the form shown in Figs. 7, 8 and 59. The tibia is much larger than the fibula and extends further distally. The astragalus has somewhat the form of a rhomboid plate which runs dorsally from the fibular side toward the tibial side on the plantar surface. The calcaneus is rather small and is in direct line with the long axis of the fibula but in a plane lying further plantarwards. The navicular is in a direct line with the astragalus. Its tibial edge lies near the lower end of the tibia. The three cuneiform bones are proportionately broader and thicker than in the adult skeleton. The cuboid is in direct line with the calcaneus. The metatarsals lie less spread apart than at an earlier stage, Figs. 5 and 6. The first phalanx has developed in all of the toes, and in the second toe, the second phalanx as well. At the region of the phalangeal joints there is a swelling of the blastemal tissue.

If now these figures be compared with Figs. 9 and 10, which show the foot of an embryo of 20 mm., the most noticeable change will be seen in the astragalus. This has become considerably thicker. It extends further than the calcaneus. Between the tibia and the navicular it has so increased in size that the foot is bent toward the fibular side. A much greater interval than in Embryo XVII, Figs. 7, 8 and 59, exists between the two bones.

The calcaneus has extended considerably in length both in a proximal and in a distal direction. The cuneiform bones are becoming crowded together. The cuboid is larger than in XVII. The phalanges are at a similar stage of development. The joints between the metatarsals and phalanges are surrounded by a mass of dense tissue, while the tissue of the joints themselves is of a light texture and resembles prochondrium.

In Embryo CXLV, *length 33 mm.*, Figs. 11 and 12, the process of cartilage formation has given rise to structures which resemble adult bones. The tibia has greatly expanded at its distal extremity and now articulates directly with the fibula. These two bones in turn articulate with the well-developed superior articular process of the astragalus. The malleolar process of the tibia is larger and extends further distal than that of the fibula. In an embryo of a corresponding age, however, Schomburg shows that the fibula extends further distal than the tibia. Individual variation may exist.

The astragalus exhibits perhaps more marked alterations in form than any other bone of the foot during the period when the embryo is growing from 20 to 30 mm. in length. Toward the tibia and fibula it develops a well-marked articular process. While this resembles closely the similar process in the adult it is less developed on its fibular side than it is in the adult. As Schomburg has shown the definite adult form is not reached before the fourth month. Toward the calcaneus the bone is well developed and against it exhibits the two characteristic articular surfaces. The posterior of these, compared with the adult, is relatively undeveloped. Distally the bone sends forth a rounded process to articulate with the navicular. In the material at my disposal the whole complex astragalus seems to arise from a single primary center.

The calcaneus, like the astragalus, undergoes marked changes in form during the latter part of the second and the first part of the third month of development. Toward the heel a well-marked tuberosity has made its appearance in Embryo CXLV, Figs. 11 and 12. Distally the bone extends to form a joint with the cuboid. Tibially it has developed a sustentaculum tali for articulation with the astragalus. It is still, however, short in proportion to its width as compared to the adult.

The navicular exhibits no marked changes. On its plantar side and tibial edge it shows a distinct tuberosity.

The cuneiform bones are crowded together and have their characteristic wedge shape. The internal cuneiform is the largest and extends farthest distal. The middle is the smallest.

The cuboid shows a tuberosity. The phalanges, all of which are developed, present no points of special interest.

The joint-cavities begin to develop while the embryo is growing from 25 to 30 mm. in length. As in other cases, so here the blastemal tissue in which the cartilages are developed becomes condensed at their articulating ends and about the joint, while in the region of the joint the tissue becomes less dense and finally disappears leaving a joint-cavity. In

embryos of about 30 mm. the joint-cavities of the foot are filled with a loose fibrous tissue, in embryos of 50 mm. definite cavities are to be made out. The sesamoid bones develop later than the period to which this investigation extends.

During the progress of form differentiation above described the shape of the foot is markedly altered. At the beginning of the development of the foot the tarsal and metatarsal bones lie nearly, though not quite, in the same plane as the bones of the leg, Figs. 7, 8 and 59. They are so arranged, however, that the foot is convex on its dorsal surface and concave on the plantar, and the projections of the calcaneus and astragalus serve to deepen the plantar fossa. The metacarpals spread widely apart. As differentiation proceeds the metacarpals come to lie more nearly parallel to one another and the tarsal elements become compacted in such a way as to give rise to the tarsal arch. The foot at the same time is flexed at the ankle and turned slightly outwards. The toes are flexed. Fig. 68 shows the extent of the tarsal arch in an embryo of 23 mm.

In the further development of the skeleton of the foot the various constituent structures are elaborated and the foot gradually becomes more flexed and turned toward the fibular side. Yet even in the infant the head of the astragalus is directed more inwards than in the adult. Leboucq, 82, pointed out that the first metatarsal is relatively short in the foetus and points more toward the tibial side than later.

Ossification.—This begins in the metatarsals and phalanges during the third month and is perichondral in nature. The tarsals begin to be ossified considerably later. The center for the calcaneus appears in the sixth month, that for the astragalus in the seventh month of foetal life. The ossification of the other bones begins during the first five years of life. Authorities differ as to the exact time at which the process begins in the various bones. In Quain's Anatomy the following dates are given: cuboid, at birth, external cuneiform, 1st year; internal cuneiform, 3d year; middle cuneiform, 4th year; navicular, 5th year.

I have studied the ossification in the third and fourth months of embryonic life. In an embryo about 4 cm. long, cleared according to the Schultze method, I have found centers of ossification in the 2d, 3d and 4th metatarsals, and in the terminal phalanx of the big toe of each foot. In Embryo XCVI, *length 44 mm.*, there is a very thin layer of bone being laid down about the center of the shaft of the 2d, 3d and 4th metatarsals. I have been unable definitely to determine whether or not bone has been deposited in the terminal phalanx of the big toe. In Embryo XCV, *length 46 mm.*, ossification has begun in the 2d, 3d and 4th metatarsals and in the terminal phalanges of the 1st and 2d toes; in Embryos

LXXXIV and CLXXXIV, *length 50 mm.*, it is apparent in the 2d, 3d and 4th metatarsals and in the terminal phalanges of the first three toes. In a cleared embryo, 6 cm. long, there are centers of ossification in all of the metatarsals and terminal phalanges; in one, 8 cm. long, in the first two basal phalanges as well; while in one, 10 cm. long, ossification has begun in all of the metatarsals and the basal and terminal phalanges. We may therefore conclude that ossification in the foot begins in the three central metatarsals and in the terminal phalanx of the first toe toward the end of the third month, and that it is thence extended to the other metatarsals and terminal phalanges before beginning in the basal phalanges.

For a consideration of the development of the individual bones of the foot reference may be made to the excellent paper of Schomburg, 90. The chief points in which my observations conflict with what he describes have been pointed out above. Hasselwander, 93, has recently published a good account of the ossification of the bones of the foot; and Spitz, 93, of the structure and development of the infant foot.

SUMMARY.

In general the development of the skeleton of the limb in man corresponds closely with that which is known to take place in other digitates and which has been recently admirably summarized by Braus, 94. Three stages may be recognized, a blastemal, a chondrogenous and an osseogenous. During the chondrogenous stage the chief features of form are reached which characterize the adult structure. The centers for chondrification correspond closely with those for ossification. The development throughout is fairly direct. No distinct evidences of phylogenetic structures discarded during ontogeny were found in the embryos studied.

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LIST OF ABBREVIATIONS USED IN LETTERING THE FIGURES.

A. A. Pr.—	Anterior articular process.	M. D. R.—	Membrana reuniens dorsalis.
Cæl.—	Cœlom.	N. Pr.—	Neural process.
Chd.—	Chorda dorsalis.	O. F.—	Obturator foramen.
Co.—	Coccygeal.	O. N.—	Obturator nerve.
C. Pr.—	Costal process.	Pd.—	Pedicle.
Cu.—	Cuboid.	Pch. S.—	Perichordal sheath.
C. V.—	Cardinal vein.	P. L.—	Poupart's ligament.
Der.—	Dermis.	P. A. Pr.—	Posterior articular process.
Disk.—	Intervertebral disk.	P.—	Pubis.
D. L.—	Dorsal ligament.	Rib.—	Rib.
D. M.—	Dorsal musculature.	S.—	Sacral vertebra.
F. D. M.—	Fascia of dorsal musculature.	S. Bl.—	Scleroblastema.
Fi.—	Fibula.	S. N.—	Sciatic nerve
F. N.—	Femoral nerve.	Sptm.—	Perichordal septum.
F. Pl.—	Foot-plate.	Sp. C.—	Spinal chord.
F. v. E.—	Fissure of v. Ebner.	Sp. G.—	Spinal ganglion.
H. Pr.—	Hæmal process.	Sp. N.—	Spinal nerve.
Ids. M.—	Interdiscal membrane.	Sp. Pr.—	Spinous process.
Idr. M.—	Interdorsal membrane.	T. R.—	Tendon of r. abd. muscle.
Il. Bl.—	Iliac blastema.	T.—	Thoracic.
Is.—	Ischium.	Ti.—	Tibia.
Is. A.—	Intersegmental artery.	Trap.—	Trapezius muscle.
L.—	Lumbar.	Tr. Pr.—	Transverse process.
Ls. Pl.—	Lumbo-sacral plexis.	V. L.—	Ventral ligament.
L. T.—	Ligamentum teres.	V. B.—	Vertebral body.
Myo.—	Myotome.		

DESCRIPTION OF PLATES.

PLATES I-V.

Figs. 1-12. A series of figures drawn from models made by the Born wax-plate method. Fig. 13 from an embryo cleared by the Schultze alkaline, glycerine method.

PLATE I.

Fig. 1. Skeleton of Embryo II, length 7 mm. About 20 diam. In part the reconstruction was made free-hand from drawings.

PLATE II.

Fig. 2. Right half of the distal portion of the skeleton of Embryo CLXIII, length 9 mm. 25 diam.

Figs. 3 and 4. Right half of the distal portion of Embryo CIX, length 11 mm. 25 diam. (4) lateral, (5) median, view. The prochondrium of the pubis, ilium, ischium, femur, tibia and fibula are represented by stippling.

PLATE III.

Figs 5 and 6. Distal portion of the right half-skeleton of Embryo CXLIV, length 14 mm. 25 diam. The prochondrium of the neural arches, the

vertebral bodies, the ilium, ischium, femur, tibia, fibula and of the bones of the foot is represented by stippling. The last are but slightly differentiated at this period.

PLATE IV.

Figs. 7 and 8. Dorsal and plantar views of the cartilages of the left leg and foot of Embryo XVII, *length 18 mm. 20 diam.*

Figs. 9 and 10. Lateral and median views of the distal portion of the right half of the cartilagenous skeleton of Embryo XXII, *length 20 mm. 20 diam.*

PLATE V.

Figs. 11 and 12. Median and lateral views of the distal portion of the right half of the cartilagenous skeleton of Embryo CXLV, *length 33 mm. 10 diam.* The centres of ossification of the femur, tibia and fibula are shown.

Fig. 13. Lateral view of the left leg of an embryo 5 cm. long. *5 diam.* For the sake of facilitating comparison, a mirror picture has been drawn and a technique has been used similar to that employed for illustrating the models. The centers of ossification of the ilium, femur, tibia, fibula, the three middle metatarsals and the terminal digits are shown. The position of the various structures has probably been somewhat distorted during the preparation of the specimen.

PLATES VI and VII.

Figs. 14-28. Transverse sections through the twelfth thoracic and first two lumbar vertebræ of a series of embryos. *14 diameters.* Figs. 14-16, Embryo CLXXV, *length 13 mm.;* Figs. 17-19, Embryo CCXVI, *length 17 mm.;* Figs. 20-22 Embryo XXII, *length 20 mm.;* Figs. 23-25, Embryo XLV, *length 28 mm.;* Figs. 26-28, Embryo LXXXIV, *length 50 mm.*

PLATE VIII.

Figs. 29-36. Transverse sections somewhat oblique through several vertebrae of various embryos. *14 diam.* Fig. 29, 4th sacral vertebra of Embryo CIX, *length 11 mm.;* Figs. 30-32, 1st, 2d, and 3d sacral vertebræ of Embryo X, *length 20 mm.;* Figs. 33 and 34, 1st and 2d sacral vertebræ of Embryo CCXXVI, *length 25 mm.;* Fig. 35, 2d sacral vertebra of Embryo XLV, *length 28 mm.;* Fig. 36, 2d sacral vertebra of Embryo LXXXIV, *length 50 mm.*

PLATE IX.

Figs. 37-40. Obliquely cut frontal sections through the sacral region of several embryos. *14 diam.* Fig. 37, Embryo CLXXV, *length 13 mm.;* Fig. 38, Embryo CCXVI, *length 17 mm.;* Fig. 39, Embryo CLXXXVIII, *length 17 mm.;* Fig. 40, Embryo LXXXVI, *length 30 mm.*

PLATE X.

Figs. 41-43. Sections through the coccygeal region of several embryos. *14 diam.* Fig. 41, frontal section of Embryo CLXXV, *length 13 mm.;* Fig. 42, sagittal section of Embryo CXLV, *length 33 mm.;* Fig. 43, frontal section of Embryo LXXXIV, *length 50 mm.*

FIG. 44. Diagram to show the curvature of the spinal column, the proportional lengths of the various regions, the relation of the acetabula to the sacral region and the direction of the long axis of the femur in a series of embryos 7 to 50 mm. in length, and in an adult. Each curved line represents the chorda dorsalis of an individual. The cervical, lumbar and coccygeal regions of this are represented by the heavy, the thoracic and sacral by the light portions of the line. The approximate position where a line joining the centers of the two acetabula would cut the median plane is represented at "a." For Embryo II, in which the skeleton of the leg is not yet differentiated the position of the future acetabula is deduced from Embryo CLXIII, *length 9 mm.* (See Fig. 2.)

The line passing in each instance from "a" and terminating in an arrow point represents the long axis of the femur. For Embryo II, this line is pointed toward the centre of the tip of the limb-bud. From "a" in each instance a perpendicular is dropped to a line connecting the two extremities of the sacral region. The numbers refer to the following embryos:

	Length 7 mm.
2, II.....	" 11 "
109, CIX.....	" 14 "
144, CXLIV.....	" 20 "
108, CVIII.....	" 33 "
145, CXLV.....	" 50 "
184, CLXXXIV.....	
Ad. Adult.	

PLATE XI.

FIG. 45. Longitudinal section through the center of the limb-bud of Embryo CLXIII. *14 diam.* Compare with Fig. 2.

Figs. 46-52. A series of cross-sections through the right leg of Embryo CIX, *length 11 mm.*

FIG. 53. Outline of the blastemal skeleton with the regions marked through which the sections 46-52 pass. *14 diam.* Compare with Figs. 3 and 4.

PLATE XII.

FIG. 54. Section from Embryo CXLIV, *length 14 mm.*, showing the pubic, iliac and ischial cartilages. *14 diam.*

FIG. 55. Section passing longitudinally through the femur and tibia of Embryo CLXXV, *length 13 mm.* A portion of the foot-plate is shown cut obliquely. *14 diam.*

FIG. 56. Longitudinal section through the ilium, femur, and tibia of Embryo XXII, *length 20 mm.* *14 diam.*

FIG. 57. Section through the pubis, ilium, ischium and head of the femur of Embryo CCXXVII, *length 30 mm.* The hip-joint cavity shows well. It does not extend into the region of the ligamentum teres. *14 diam.*

FIG. 58. Section through the ilium, ischium and head of the femur of Embryo LXXIX, *length 33 mm.* Calcification is beginning in the ilium.

PLATE XIII.

FIG. 59. Section through the leg and foot of Embryo XVII, *length 18 mm.* The section does not pass through the cartilage of the 1st metatarsal.

FIG. 60. Section through the pubis, ischium, femur, fibula, calcaneus, cuboid and the 4th metatarsal cartilages of Embryo LXXIV, *length 16 mm.* *14 diameters.*

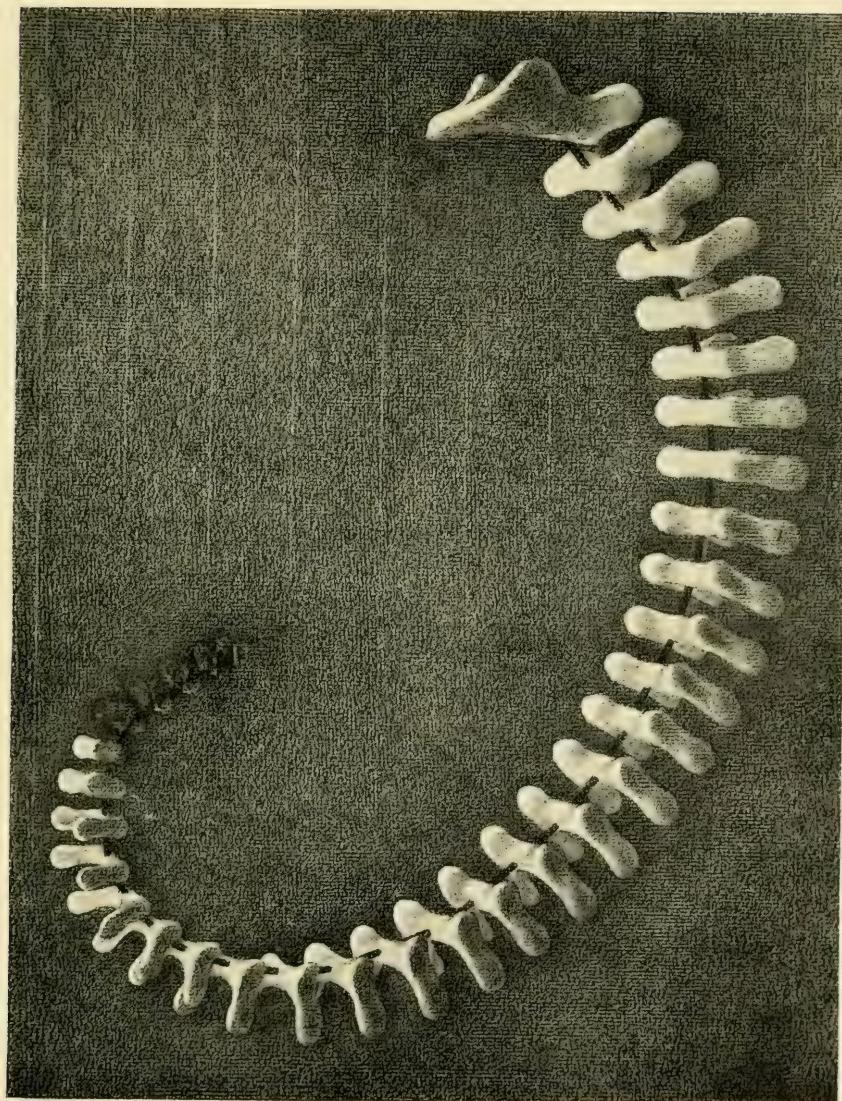
Figs. 61-65. Sections through the knee-joints of several embryos. *14 diam.*; 61, CCXXIX, *length about 20 mm.*; 62, LXXXVI, *length 30 mm.*; 63, LXXV, *length 30 mm.*; 64 and 65, CXLV, *length 33 mm.*

FIG. 66. Longitudinal section through the knee-joint, tibia and foot-plate of Embryo CLXXV, *length 13 mm.*

FIG. 67. Section through the foot of Embryo CXLIV, *length 14 mm.*

FIG. 68. Section through the foot of Embryo LVII, *length 23 mm.*

The models from which the illustrations in this article were drawn have been reproduced by Dr. B. E. Dahlgren at the American Museum of Natural History, New York, N. Y., and arrangements may be made for securing copies by applying to the Director of the Museum.



II, LENGTH 7 MM.

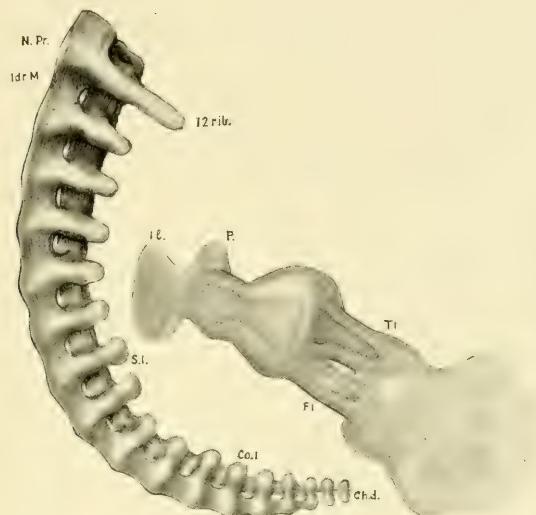


Fig. 3

CIX, LENGTH 11 MM.

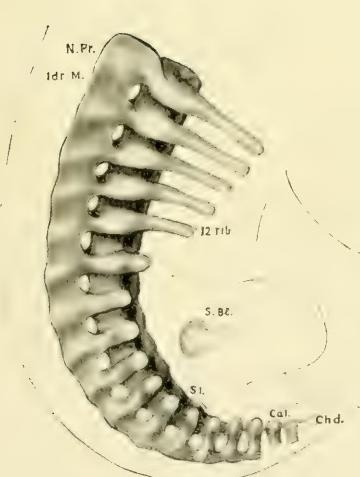


Fig. 2

CLXIII, LENGTH 9 MM.

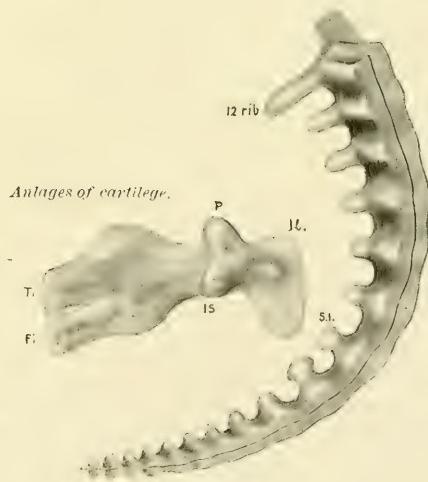
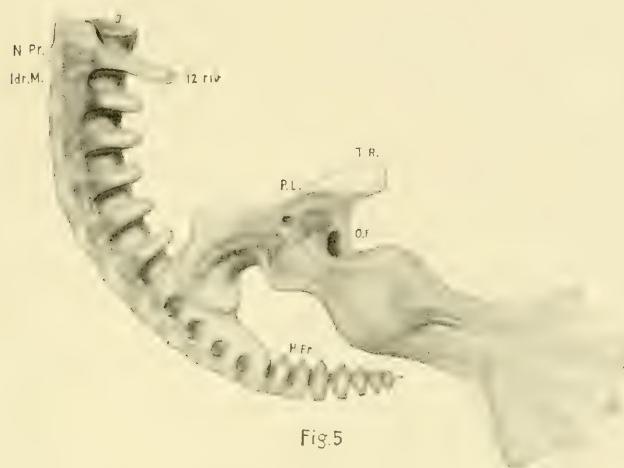


Fig. 4.

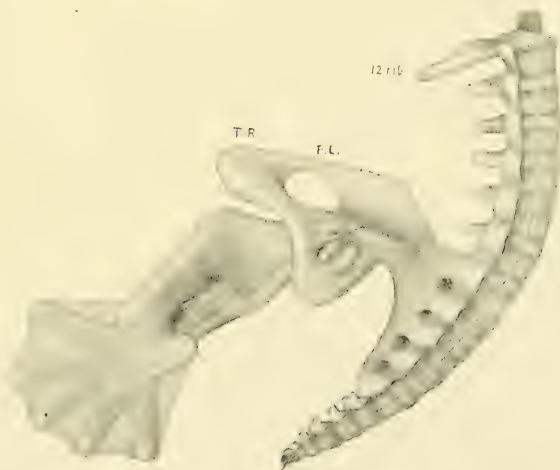
CIX, LENGTH 11 MM.

DEVELOPMENT OF THE HUMAN SKELETON
C. R. BARDEEN

PLATE III



CXLIV, LENGTH 14 MM.



CXLIV, LENGTH 14 MM.



Fig. 7

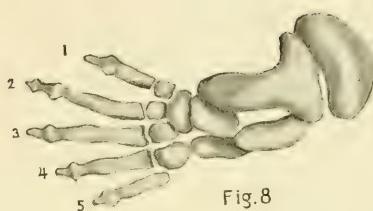


Fig. 8

XVII, LENGTH 18 MM.

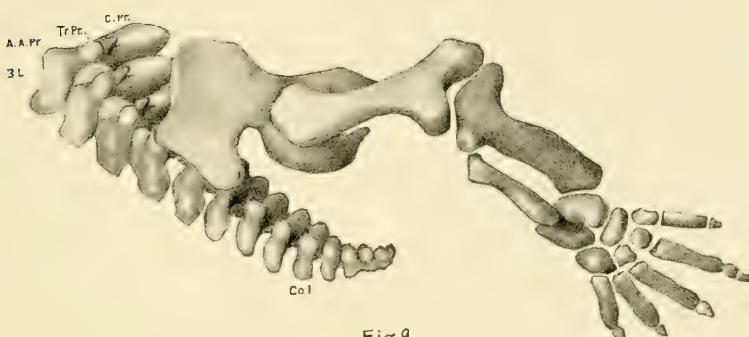


Fig. 9

XXII, LENGTH 20 MM.

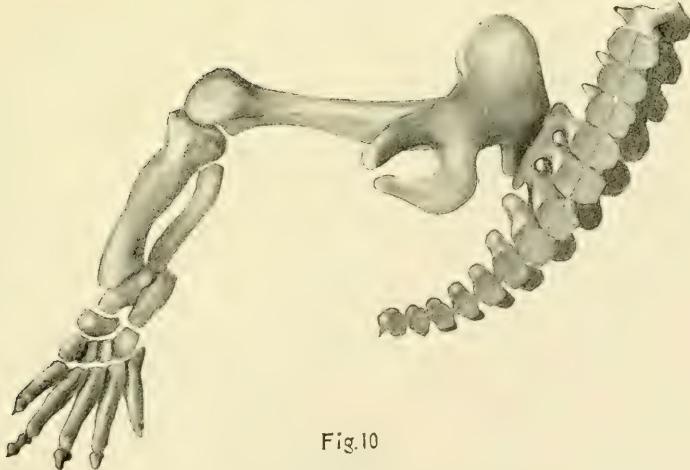


Fig. 10

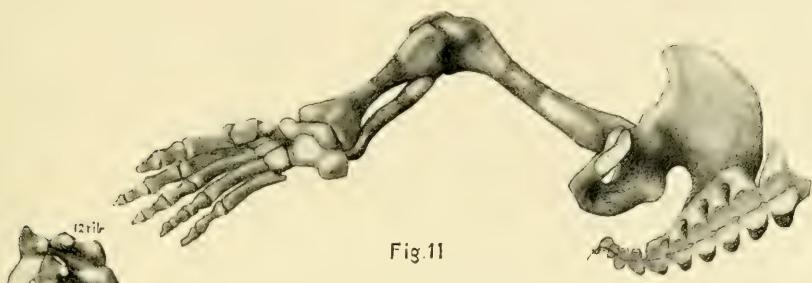
XXII, LENGTH 20 MM



DEVELOPMENT OF THE HUMAN SKELETON

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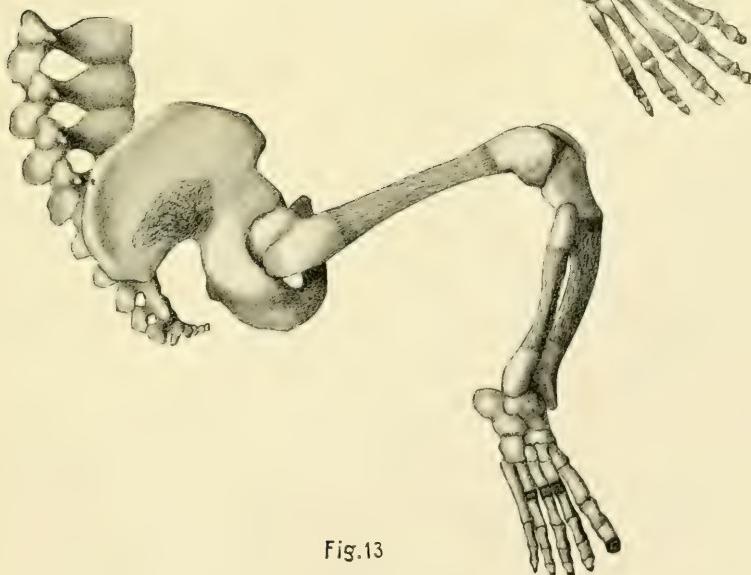
PLATE V



CXLV, LENGTH 33 MM.

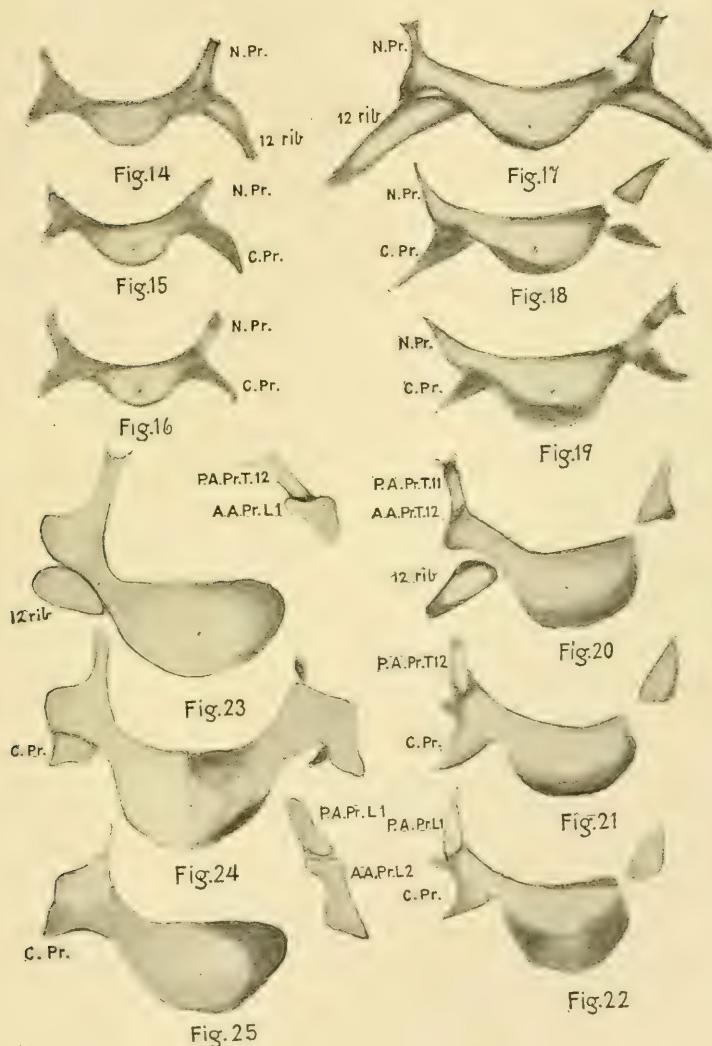


CXLV, LENGTH 33 MM.



LXXXIV, LENGTH 50 MM.





DEVELOPMENT OF THE HUMAN SKELETON

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PLATE VII

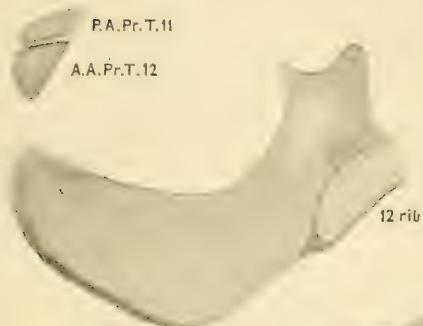


Fig.26

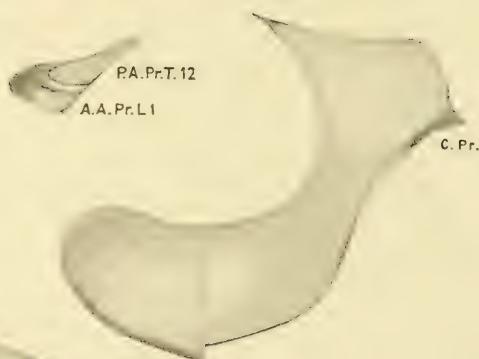


Fig.27

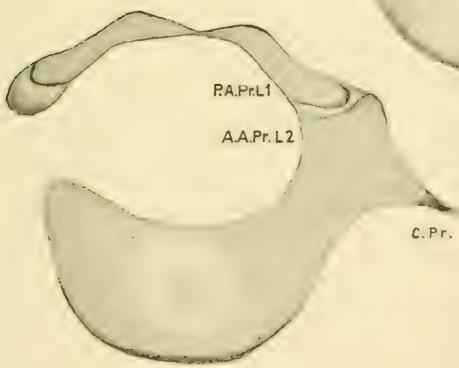
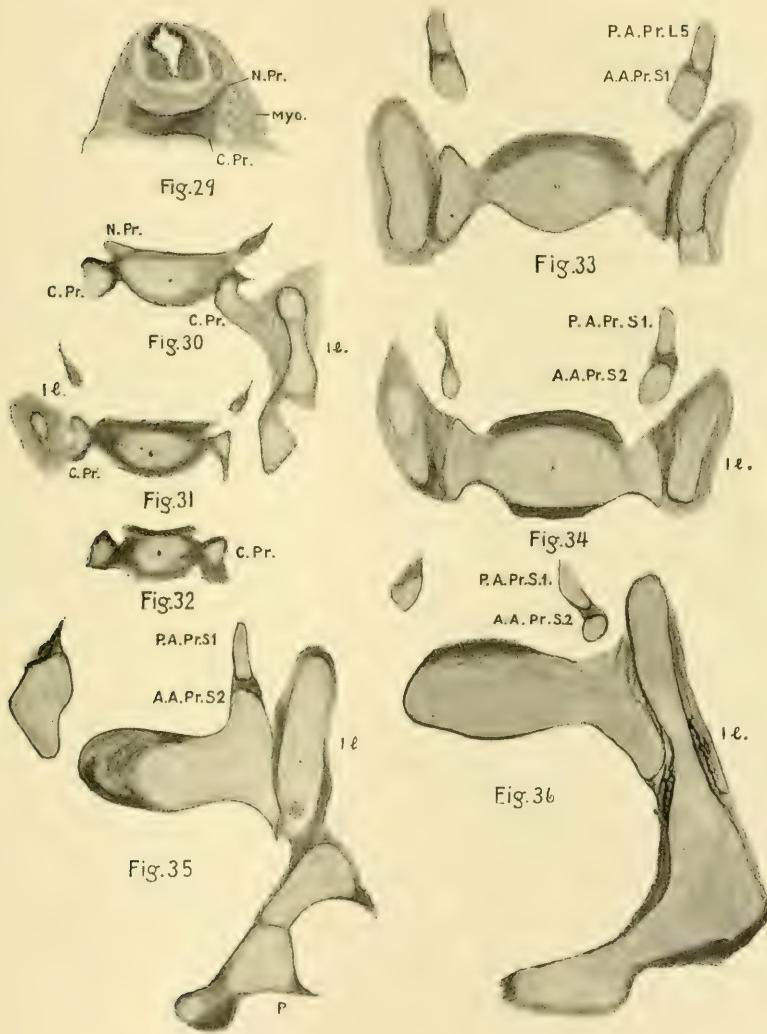


Fig.28



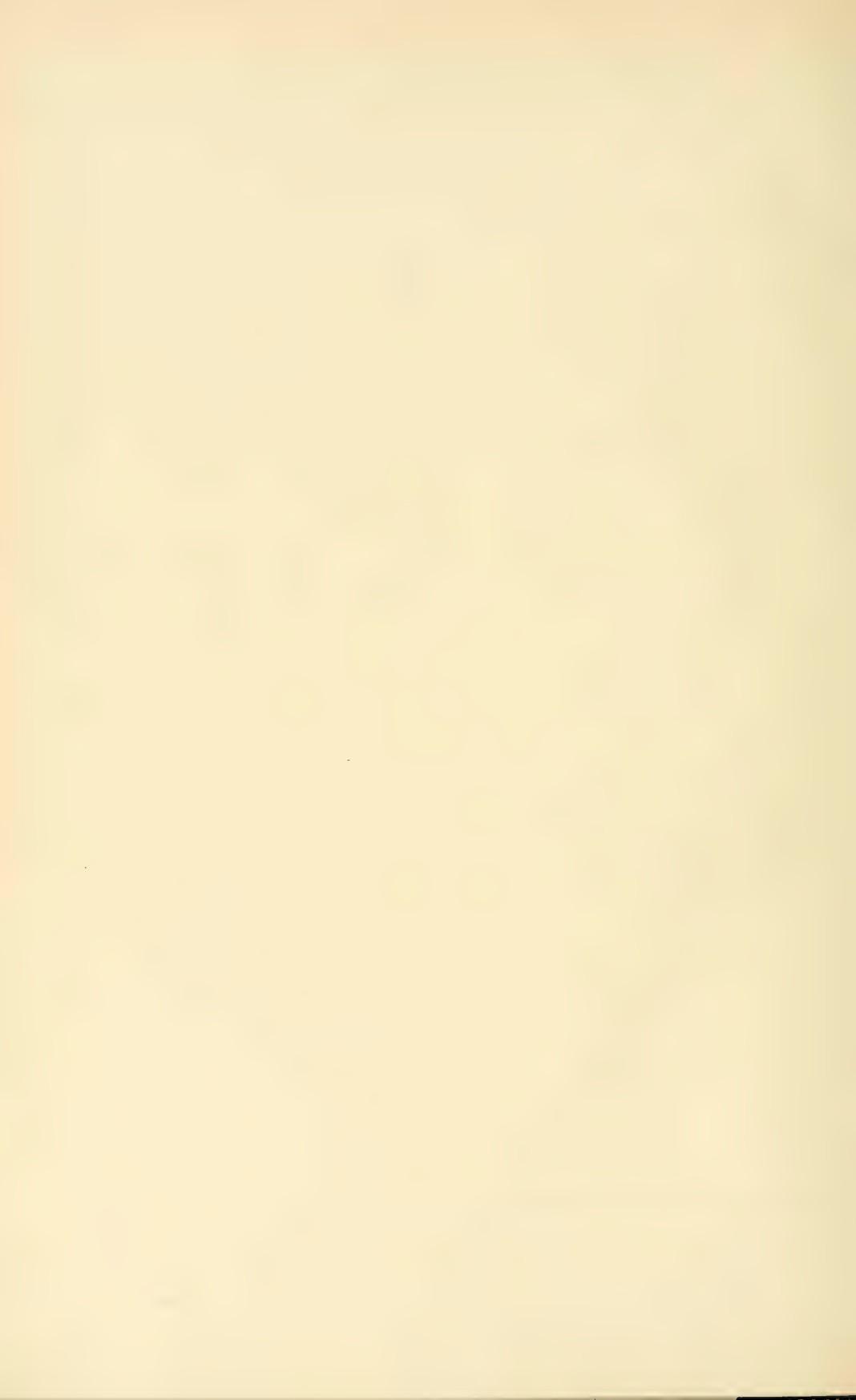




Fig. 37

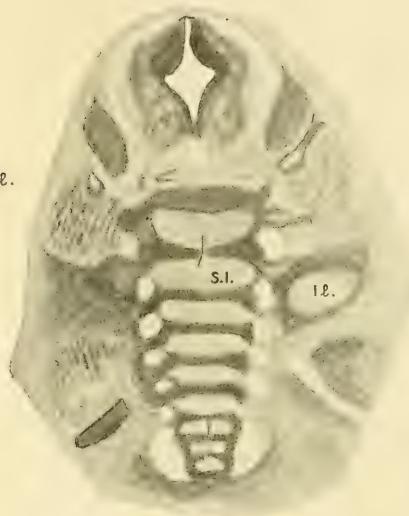


Fig. 38

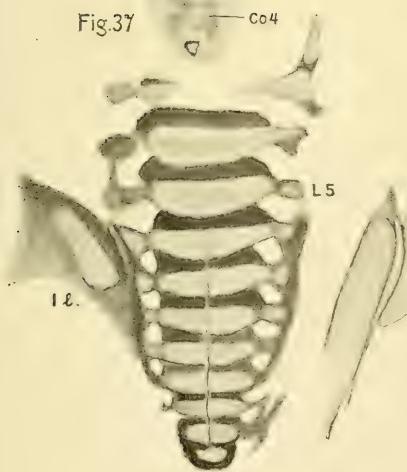


Fig. 39

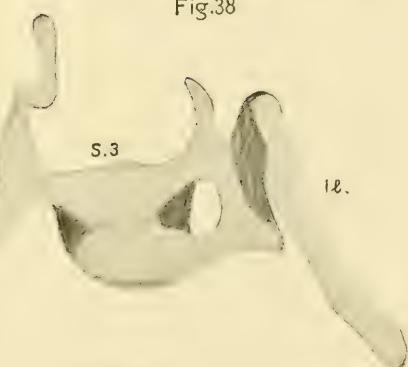


Fig. 40



Fig. 44



Fig. 41

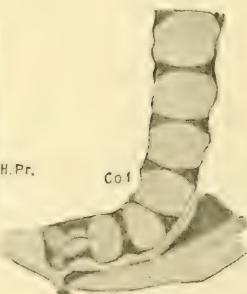


Fig. 42

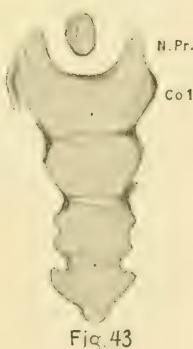


Fig. 43

DEVELOPMENT OF THE HUMAN SKELETON

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PLATE XI

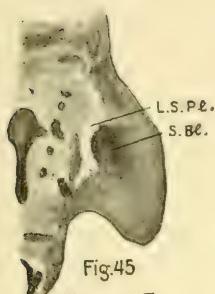


Fig.45

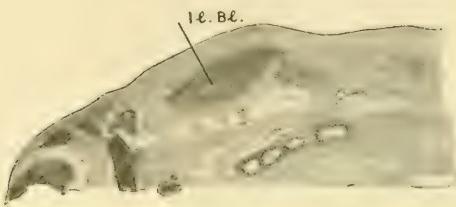


Fig.46



Fig.50

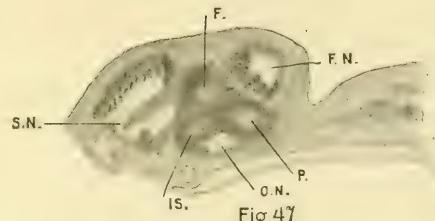


Fig.47

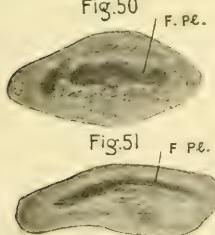


Fig.51



Fig.52



Fig.48

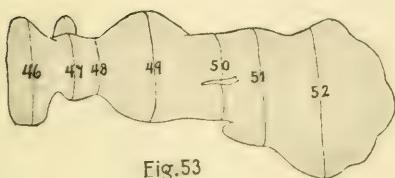


Fig.53



Fig.49

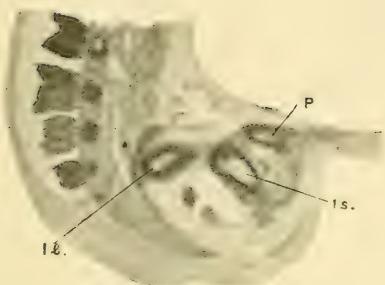


Fig. 54



Fig. 55

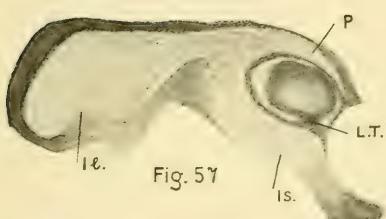


Fig. 57

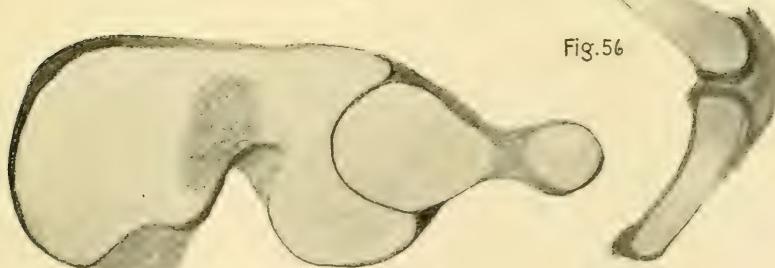


Fig. 56

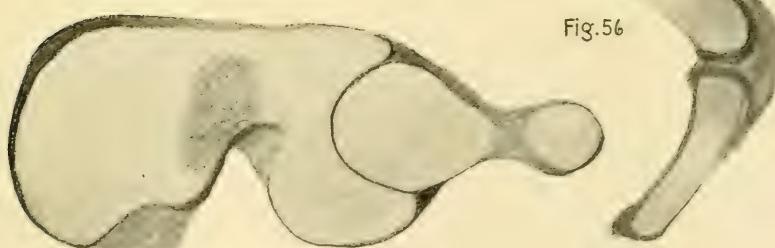


Fig. 58

DEVELOPMENT OF THE HUMAN SKELETON

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PLATE XIII

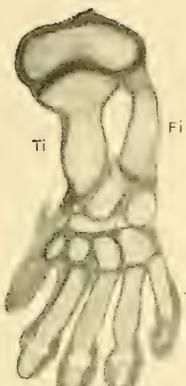


Fig. 59

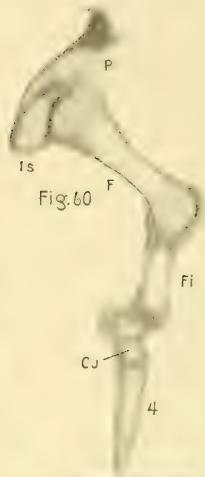


Fig. 60



Fig. 61

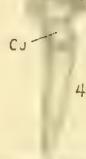


Fig. 62



Fig. 63



Fig. 64

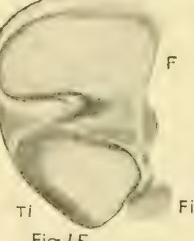


Fig. 65



Fig. 66



Fig. 67

Fig. 68.

A COMPOSITE STUDY OF THE SUBCLAVIAN ARTERY IN MAN.

BY

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WITH 7 FIGURES AND 18 TABLES.

Several years ago Hitzrot¹ made a study of the axillary artery based upon records made in the Anatomical Laboratory of the Johns Hopkins University. To supplement this, the following study of the subclavian artery was made at the suggestion of Dr. Harrison. The clinical features relating to the artery are given in another article.²

That there is need for further data concerning the ramifications of this artery is apparent when the accompanying figures, taken from a number of universally recognized authorities, are compared. From them it is seen, that, while certain branches such as the vertebral and internal mammary are represented in the same manner by all, there is the widest divergence with regard to the other branches.

The records which underlie the present study were made by myself, from the dissections by students of anatomy, upon Bardeen's charts.³ Dissections from 129 subjects are recorded, 60 from the left side of the body and 69 from the right side. Some of these records are complete to the minutest detail; nearly all give the origin of the main branches; while a few are incomplete, giving only the subclavian artery and some of its branches, or only a few branches without the subclavian artery. The distribution of the vertebral artery inside the skull was not worked out, because many of the cadavers were not obtained until after the brain had been removed. The distribution of the internal mammary artery was worked out completely in but 28 cases because of the removal of the sternum at the autopsy in the others.

¹ Hitzrot, Johns Hopkins Hospital Bulletin, Vol. XII, 1901.

² Bean, Johns Hopkins Hospital Bulletin, Vol. XV, 1904.

³ Bardeen, Outline Record Charts, Johns Hopkins Press, Baltimore, 1900.
AMERICAN JOURNAL OF ANATOMY.—VOL. IV.

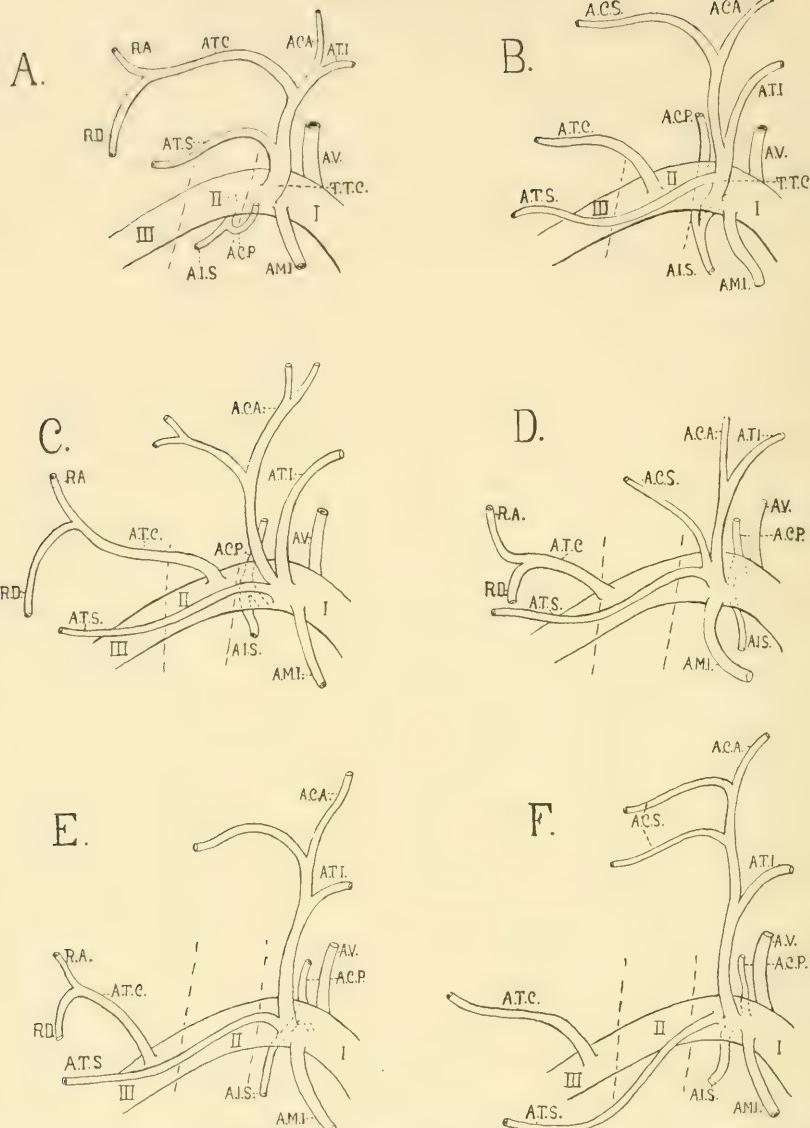


FIG. 1. Branches of the subclavian artery according to different authors. *A*, according to Quain, Testut and Gray; *B*, according to Henle; *C*, according to Tiedemann; *D*, according to Spaltheholz and Toldt (B. N. A.); *E*, according to Gegenbaur; *F*, according to Sappey.

The lettering on all the figures is alike and as follows: I, II and III, the three parts of the subclavian artery; *A. V.*, arteria vertebralis; *A. M. I.*, arteria mammaria interna; *T. T. C.*, truncus thyreo-cervicalis; *A. T. I.*, arteria thyroidea inferior; *A. T. S.*, arteria transversa scapula; *A. T. C.*, arteria transversa colli; *R. A. T. C.*, ramus ascendens transversa colli; *R. D. T. C.*, ramus descendens transversa colli; *A. C. S.*, arteria cervicalis superficialis; *A. C. A.*, arteria cervicalis ascendens; *T. C. C.*, truncus costo-cervicalis; *A. I. S.*, arteria intercostalis suprema; *A. C. P.*, arteria cervicalis profunda; *C. T.*, common trunk.

While numerous variations in the origin and distribution of the branches of the artery are observed in my study, it is nevertheless possible to classify the cases, for they are found to fall naturally into a number of distinct types. In section A of this work it is proposed to describe these types. This will be followed in section B by a description of the origin and distribution of the individual branches, while in section C the results of the present study will be discussed in their relation to the previous work upon the subject, and illustrative tables will be appended.

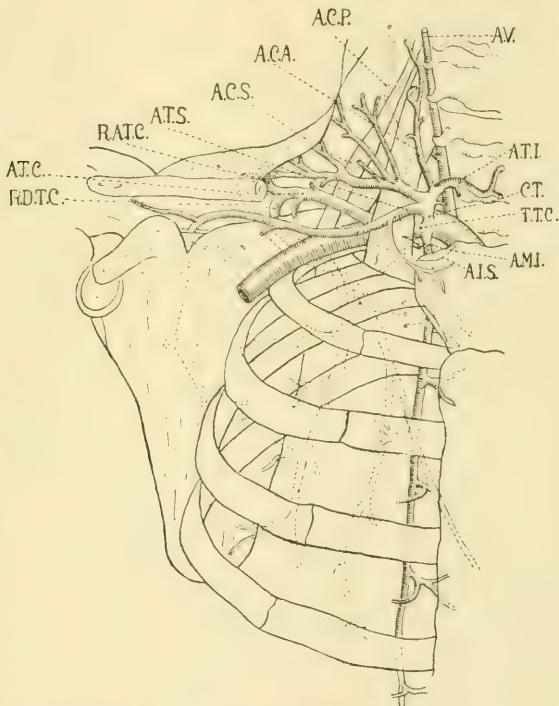


FIG. 2. Type I, occurring in 30% of the specimens, 22% on the right side, and 8% on the left side of the body. For index to lettering see Fig. 1.

The three divisions of the subclavian artery referred to throughout this work are: Part I, that portion medial to the scalenus anticus muscle; Part II, posterior to it; and Part III, lateral to this muscle.

The records given are from 74 male Negroes, 16 female Negroes, 21 male Caucasians and 3 female Caucasians. The race and sex are not determined in 15 subjects. The Negroes are the American variety, and possibly all of them have a trace of the Caucasian mixed with the Negro, the proportion in any case being uncertain.

SECTION A.—TYPES OF RAMIFICATION.

The mode of ramification of the subelavian artery is found to be divided into five types, depending upon the origin of the large branches. The distribution of these branches is practically the same in all cases.

Type I (Fig. 2) occurs in 30% of the cases classified, 22% being on the right side of the body and 8% on the left side. In this type the vertebral and internal mammary arteries rise from Part I; the inferior thyroid and suprascapular arteries rise from a common trunk which comes from Part I, and between these two arteries rises the superficial

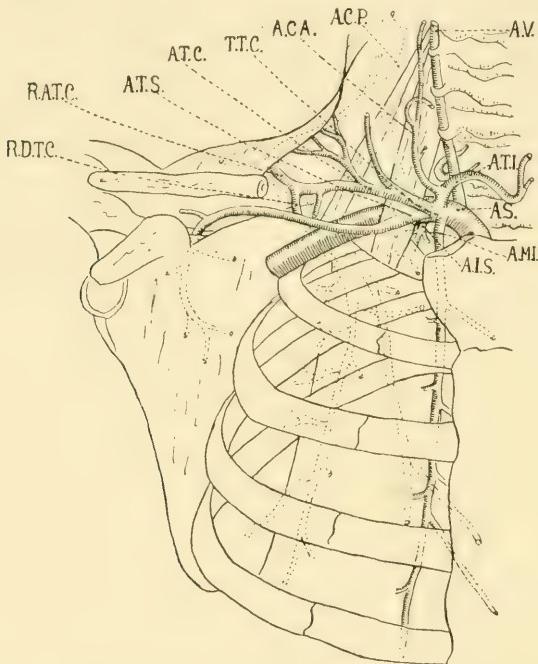


FIG. 3. Type II, occurring in 27% of the specimens, 22% on the left side, and 5% on the right side of the body. For index to lettering see Fig. 1.

cervical artery; the ascending cervical artery rises from the inferior thyroid; and the transverse cervical artery and the costo-cervical trunk rise from Part II. Each of the branches often has a separate origin. There are in this type 19 male negro subjects, 2 female negro subjects, 5 male white subjects, 2 female white subjects, and 4 subjects in which the sex and race are not determined.

Type II (Fig. 3) is found in 27% of the cases classified, 22% being on the left side of the body and 5% on the right side. The vertebral

and internal mammary arteries rise from Part I; the inferior thyroid, suprascapular and transverse cervical arteries rise from a common trunk which comes from Part I, and is known as the thyroid axis; the superficial cervical artery is absent, its place being taken by small branches from the transverse cervical artery; the ascending cervical artery rises from the inferior thyroid artery, as it does in practically all the cases of all the types; and the costo-cervical trunk rises from Part II. The internal mammary artery rises from the thyroid axis five times in this type—four times in infants—showing a bunching of the branches.

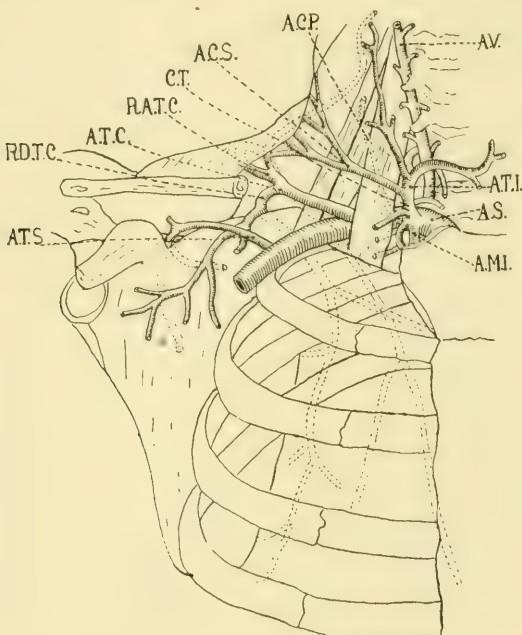


FIG. 4. Type III, occurring in 22% of the specimens. For index to lettering see Fig. 1.

There are in this type 18 male negro subjects, 2 female negro subjects, 3 male white subjects, 1 female white subject, and 1 subject in which the sex and race are not determined. Types I and II are the representative types for the right and left sides of the body respectively. Cf. Fig. 7, A and B, pp. 314 and 315.

Type III (Fig. 4) with slight variations occurs in 22% of the cases classified, 25 times in all, 13 on the right side of the body and 12 on the left side. The vertebral and internal mammary arteries, and the costo-cervical trunk rise from Part I in this type and in the two remaining

types. The inferior thyroid artery rises from Part I, the transverse cervical artery rises from Part II, and the suprascapular artery rises from Part III, or from the axillary artery (9 times). This type may be considered a subtype of the first, Type I, showing the extreme separation of the origin of the branches and no bunching. There are in this type 11 male negro subjects, 4 female negro subjects, 7 male white subjects, and 3 subjects in which the sex and race are not determined.

Type IV (Fig. 5) is found in 12% of the cases classified, 14 times in all, present in equal number on each side of the body. The inferior thy-

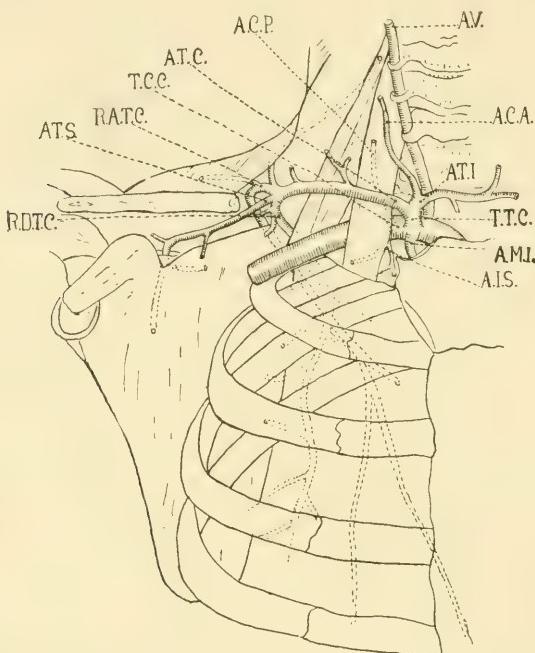


FIG. 5. Type IV, occurring in 12% of the specimens. For index to lettering see Fig. 1.

roid artery rises with a common trunk from Part I. From the common trunk rise the suprascapular and transverse cervical arteries. This type may be considered as a subtype of the second, Type II, in which the branches are often bunched. There are in this type 4 male negro subjects, 3 female negro subjects, 3 male white subjects, and 4 subjects in which the sex and race are not determined.

Type V (Fig. 6) occurs in 10% of the cases classified. The inferior thyroid and superficial cervical arteries rise by a common trunk from

Part I; the suprascapular artery rises from the internal mammary artery. The type is of interest from this fact and because of its frequent occurrence in the cases studied.

SECTION B.—DESCRIPTION OF THE INDIVIDUAL BRANCHES.

In its origin the *vertebral artery* is the most constant of all the branches of the subclavian artery. It arises in every case, with three exceptions, from the posterior and superior aspect of Part I, and is the first and largest branch. It is associated with other arteries in its origin.

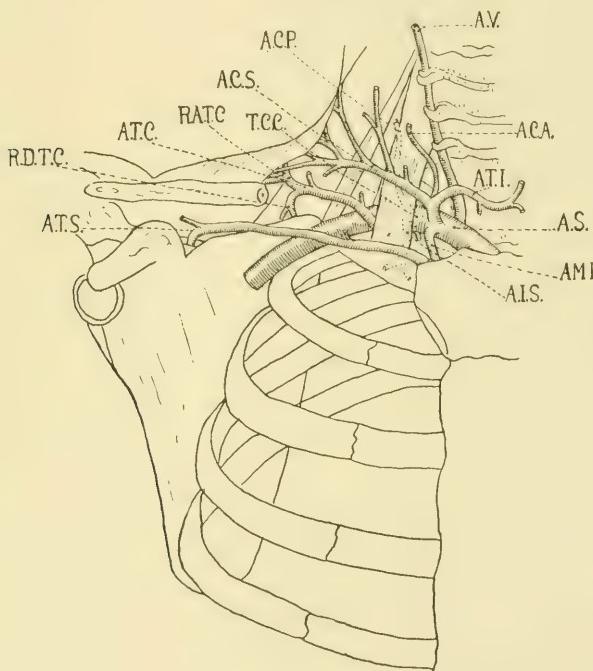


FIG. 6. Type V, occurring in 10% of the specimens. For index to lettering see Fig. 1.

from a common trunk but four times, with the inferior thyroid three times, and the thyroid axis once. It comes from the arch of the aorta between the origin of the left common carotid and the left subclavian arteries three times. In one case on the right side the vertebral artery is double, two small arteries arising from Part I and entering the 6th vertebral foramen together. The artery enters the 4th foramen once; the 5th, 4 times; the 6th, 88 times; and the 7th, 4 times. Tiedemann states that this artery enters any one of the vertebral foramina from the

1st to the 7th, most frequently the 6th, and most infrequently the 7th. He also mentions a double vertebral artery, one arising from the inferior thyroid artery, the other from the subclavian, uniting at the 4th cervical vertebra. Quain gives a chart of a similar double vertebral artery.

The internal mammary artery arises alone from Part I in 80% of the cases, and is associated with other arteries by origin in a common trunk in 20% of the cases, arising with the thyroid axis in 10% of the latter, with the suprascapular in 10% of them, and with the transverse cervical and suprascapular once. The distribution of the artery is worked out in minute detail only 28 times. A lateral thoracic artery is found five times in the 28. It is as large as the internal mammary, is derived from the latter close to its origin from the subclavian, and passes between the parietal pleura and the ribs along the anterior axillary line, sending branches into the intercostal spaces from the 1st to the 9th, and losing itself in one of these spaces or in the diaphragm. A lateral thoracic artery is mentioned by Quain, Tiedemann, Henle, and other anatomists, but is given as a very rare anomaly. Intercostal branches come from the internal mammary as single arteries posterior to the intercostal spaces, sending one branch to the superior part and another to the inferior part of the spaces; or they arise posterior to the costal cartilages, sending a branch above and one below the adjoining rib; or there are two intercostal branches to each space, one below the rib above it, the other above the rib below it. Any two or all three of these arrangements may be found on one side of a subject. In 54% of the subjects there are two branches to each intercostal space, in 46% only one.

*The thyroid axis*⁴ is found as shown in Type I, Fig. 2, in 30% of the cases, 22% of these being on the right side of the body, and 8% on the left side. It is present as shown in Type II, Fig. 3, in 27% of the cases, 22% of these being on the left side of the body, and 5% on the right side. Quain and Gray give Type II as normal. Tiedemann, Henle, Gegenbaur, Sappey, Testut and other French and German anatomists give Type I as the most frequent.

*The inferior thyroid artery*⁵ arises as shown in Type I in 35% of the subjects; it arises from Part I as a single branch in 33% of the subjects, and as shown in Type II, in 32% of the subjects.

*The suprascapular artery*⁶ arises as shown in Type I in 36% of the subjects; in Type II in 34%, and from the subclavian alone as a single branch in 30% of them.

This artery is absent 4 times, double 3 times, and very small 4 times. The long thoracic artery arises from it once.

⁴Table 2, p. 318.

⁵Table 3, p. 318.

⁶Table 5, p. 319.

*The transverse cervical artery*⁷ arises from Part II in 39% of the subjects, from Part I in 36% of the subjects (alone or with the thyroid axis), and from Part III, or from the axillary artery in 25% of them. Quain gives the most frequent origin of the transverse cervical artery from the thyroid axis, dividing into the posterior scapular and superficial cervical; the next in frequency being the posterior scapular from Part III and the superficial cervical from the thyroid axis; the least frequent mode of origin being from Part III, and dividing into posterior scapular and superficial cervical arteries. We found the following approximately:

The transverse cervical artery arises on the right side from Part II, dividing into ascending [superficial cervical (?)] and descending (posterior scapular) rami, and on the left side from the thyroid axis, dividing into ascending and descending rami, having previously given off the superficial cervical artery. The ascending ramus of the transverse cervical artery arises lateral to the levator scapulæ muscle, and, dividing almost immediately, sends one branch parallel to the *superior* lateral border of the trapezius and beneath it to the occiput. The other branch passes parallel to the *inferior* lateral border of the same muscle and beneath it to the level of the seventh thoracic vertebra, sending a large branch to the rhomboid muscles. The descending ramus follows the prescribed course of the posterior scapular artery as given in English and American text-books. The relation of the two sides of the body with reference to the origin of the transverse cervical artery shows the two sides alike in 29 subjects, unlike in 13. In 10 of the latter the artery arises from Part II on the right side, and from the thyroid axis on the left side.

*The superficial cervical artery*⁸ is considered to be a branch that passes from the transverse cervical artery in 60% of the subjects, from the inferior thyroid artery in 22% of the subjects, and from the suprascapular artery in 18% of the subjects, terminating just beneath the lateral border of the trapezius muscle. The artery is more commonly a number of small branches arising along the transverse cervical artery as it traverses the neck. The ascending ramus of the transverse cervical artery is described by some anatomists as the superficial cervical artery (Quain).

*The costo-cervical trunk*⁹ a small short artery, arises from Part I in 90% of the subjects; from Part II in 9% of the subjects, and from

⁷ Tables 6, 7, and 8, p. 319.

⁸ Table 9, p. 320.

⁹ Tables 10 and 11, p. 320.

Part III in 1% of them, dividing almost immediately into the superior intercostal and the deep cervical arteries. The origin from Parts II and III is on the right side of the body in all cases.

*The superior intercostal artery*¹⁰ arises on the right side of the body from the costo-cervical trunk in 41% of the subjects; from Part II in 10% of the subjects, and from Part I in 3% of them. It arises on the left side of the body from the costo-cervical trunk in 38% of the subjects, and from Part I in 8% of them.

*The deep cervical artery*¹¹ arises from the costo-cervical trunk in 83% of the subjects; from the subclavian artery in 13% of them, and from the inferior thyroid in 4% of them. It passes above the first rib in 82% of the subjects, and below it in 18% of them. The distribution of the deep cervical artery varies in inverse proportion to that of the ascending cervical and the superior intercostal arteries.

SECTION C.—DISCUSSION.

First.—We have demonstrated that the branches are arranged in a different manner on the two sides of the body. Fig. 7 shows this.

This figure represents the most usual arrangement of the branches of the subclavian artery as found on each side of the body, the difference between the two sides of the body being chiefly in the origin of the transverse cervical artery.¹² The type shown on the right side of the body occurred in 51% of all the cases classified on that side. The type shown on the left side of the body occurred in 55% of all the cases classified on that side. The distribution of the anterior branches is put on the right side of the body, and that of the posterior branches on the left side of the body in this figure.

Second.—The number of branches arising from Part II on the right side is more than double those from the same part on the left side, counting all cases. This is due to the origin of the transverse cervical artery and occasionally (11 times) the superior intercostal artery from Part II on the right side.¹³

Third.—The relation of the branches to age discloses the apparent abnormality of infantile subclavian arteries. There are 23 infant subjects worked out, 17 male negro, 4 female negro, and 2 male white. No two subjects show the same arrangement, all being irregular.

¹⁰ Table 10, p. 320.

¹¹ Table 11, p. 320.

¹² Tables 12, 13, 14, 15, and 16, pp. 320, 321, 322, and 323.

¹³ Tables 5, 10, 13, and 14, pp. 319, 320, and 321.

Two striking features are noticed. In the first place there seems to be a tendency for the branches to be bunched from Part I. The internal mammary artery arises 4 times with the thyroid axis, and the suprascapular artery arises twice from the internal mammary artery. In the second place the suprascapular artery is small in five cases, and does not extend beyond the suprascapular notch in these cases, its place being taken by the dorsal scapular artery. There are 2 other cases with inosculation around the neck of the scapular between these two arteries, the suprascapular being like a continuation of the dorsal scapular artery. Knowledge of the previous condition of the subject as to age, habits, and family history, and dissection of subjects that had died at the ages of 1, 5, 10, 15, 20, and 25 years, etc., would be of value in studies similar to this one.

Fourth.—There are 74 male negro and 16 female negro subjects, and 21 male white and 3 female white subjects from which records were made. In 15 subjects the race and sex are not determined. In view of the small number of female and white subjects, the relation of sex and race will hardly admit of discussion. Many of the subjects are mulattoes, or mixed bloods. The number of anomalies, variations, and queer types is uncommonly large.

May we not explain the occurrence of this large number of abnormalities by the well-known biological law that hybrids tend toward variation? The question is an open and an interesting one.

Fifth.—Free anastomoses by a definite arterial trunk were found in connection with the suprascapular, deep cervical and superior intercostal arteries. The suprascapular artery *inosculates* with the dorsal scapular artery posterior to the neck of the scapula, 17 times, or in 16% of all cases. The trunk was from 2 to 5 mm. in diameter. The superior intercostal artery anastomoses with the superior aortic intercostal artery 31 times, in every case where it is looked for. The anastomosis is found to take place:

At the second intercostal space	16 times
At the third intercostal space.....	10 times
At the fourth intercostal space.....	5 times

The trunk is very small, only a minute tube in some cases. The deep cervical, "profunda cervicis," anastomoses with the "princeps cervicis" from the occipital in 11 cases, 10% of all. The trunk is of good size, frequently about 5 mm. in diameter.

Sixth.—Anomalies when present are found as a rule on each side of the same subject. The most frequent anomaly met with in the dissec-

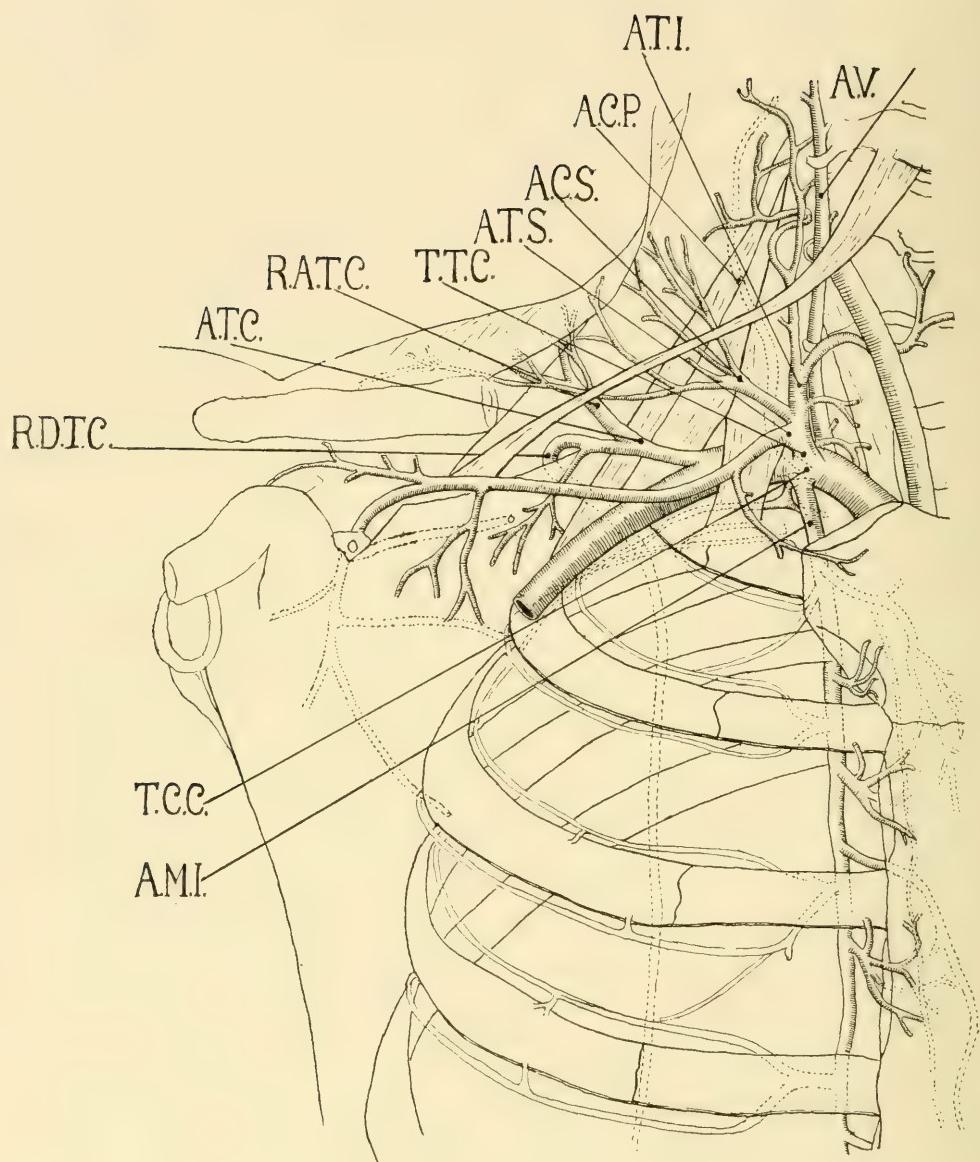


FIG. 7-A. The right subclavian artery. See Fig. 1 for index to lettering.

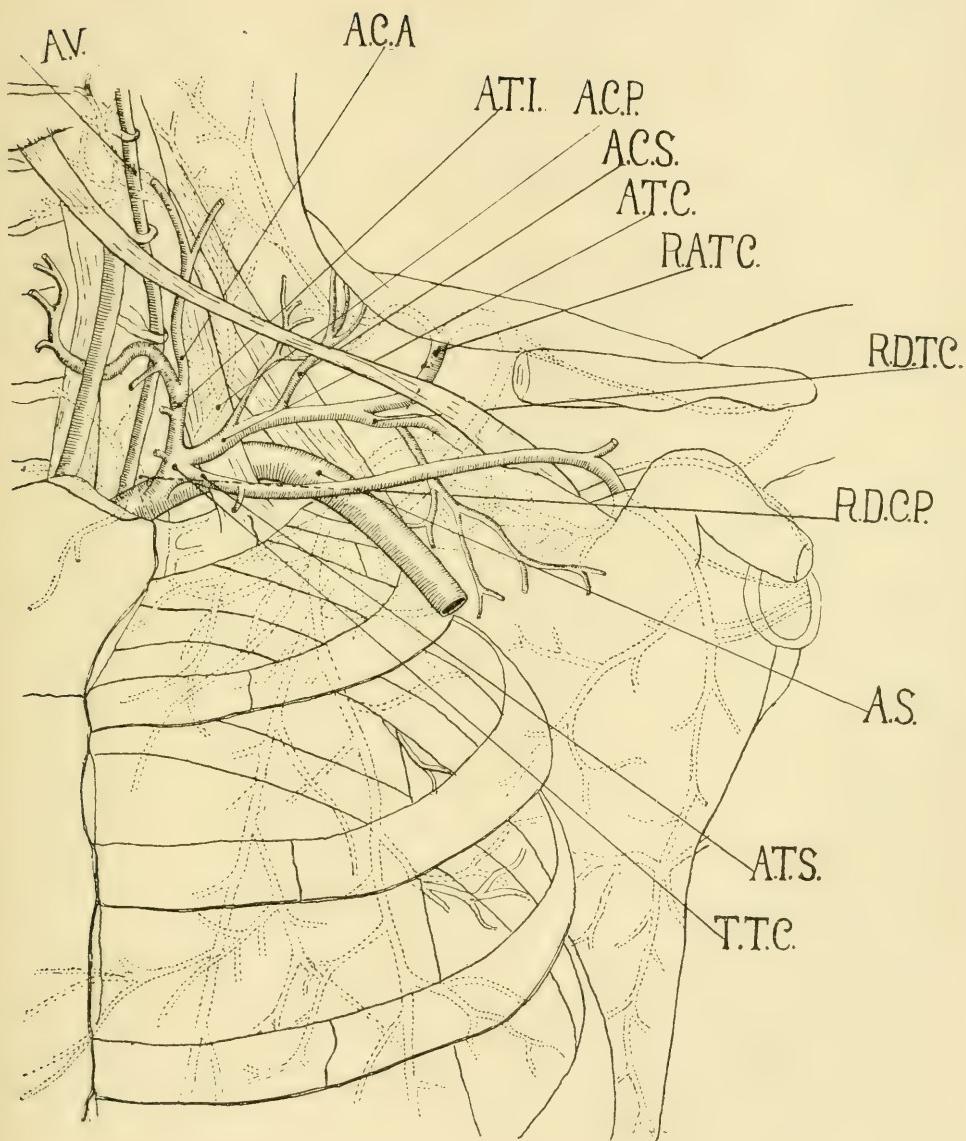


FIG. 7-B. The left subclavian artery of the same body as Fig. 7-A. See Fig. 1 for index to lettering.

tions is the suprascapular artery arising from the internal mammary artery (see Fig. 6). This occurs 12 times in 104 cases (practically 12%). Quain¹⁴ found the same anomaly 4 times in 264 dissections of the subclavian artery (about 1%). Arthur Thomson¹⁵ found it 9 times in 544 cases (less than 2%). Another anomaly is found in connection with the internal mammary artery. The latter divides into two branches a few cm. from its origin, one of which takes the normal course of the internal mammary artery, while the other follows the anterior axillary line between the ribs and pleura, terminating at the diaphragm in two cases, at the fourth rib in three other cases. This branch is as large as the ordinary internal mammary artery, and sends branches into the intercostal spaces just as that artery does. This "lateral thoracic artery" is present 5 times in 28 cases (18%) that are carefully worked out. Anatomists mention this anomaly, but consider its occurrence much rarer than our findings indicate. Another important anomaly is observed twice. The anomaly is in the trunks arising from the arch of the aorta. The first trunk divides immediately at the aorta into the two common carotid arteries. The second trunk is the left subclavian artery. The third trunk is the right subclavian artery. This arises from the distal part of the aortic arch on a level with the fourth thoracic vertebra, and passes posteriorly between the oesophagus and the vertebral column to its usual place on the right side. The right recurrent laryngeal nerve passes directly to its distribution, without looping around the subclavian artery. The pneumogastric and phrenic nerves occupy their usual places and relations. This anomaly has been reviewed by Gotthold Holzapfel,¹⁶ who collected 200 cases from the literature, including 4 of his own (1 in an animal). He concludes that this anomaly occurs 6 times in every 1000 cases. Quain and other anatomists fixed the ratio at 4:1000. Tiedemann gives the ratio 8:1000, nearly 1%. The middle thyroid artery, "Thyroidea Ima," another anomaly, is found three times. It arises from the innominate artery, and, passing to the median line, supplies the lower lobes of the thyroid gland and the isthmus. Wenzel Gruber¹⁷ records 125 anomalies of this kind, and concludes that the artery rises most frequently from the innominate artery, but also not infrequently comes from the aorta and the common carotid artery. He found it 16 times in 100 consecutive dissections.

¹⁴ Quain, *Commentaries on the Arteries*, London, 1844.

¹⁵ Thomson, *Second Report of the Collective Investigation of the Anatomical Society of Great Britain and Ireland. Journal of Anatomy and Physiology*, London, Vol. XXVI, p. 78.

¹⁶ Holzapfel, *Anatomische Hefte*, XII, I part, p. 373 (1897).

¹⁷ Gruber, *Virchow's Archiv*, Vol. 54, p. 445.

SUMMARY.

I. The branches of the subclavian artery differ in their origin on the two sides of the body, the most frequent arrangement being similar to Type I on the right side, and Type II on the left side.

(a). The thyroid axis, dividing into the suprascapular, transverse cervical, and inferior thyroid arteries, is not normal, except on the left side.

(b). The transverse cervical artery and the costo-cervical trunk arise from the second part of the subclavian artery more frequently on the right side than on the left side.

(c) The superficial cervical artery is of infrequent occurrence, and is found more often on the right side. See Type I.

(d). The transverse cervical artery terminates by dividing into ascending and descending rami, the latter being commonly called the posterior scapular artery. The former divides underneath the trapezius muscle and supplies the upper and middle part of the back.

(e). There is a tendency in the branches of the subclavian artery to bunch themselves in their origin on the left side, whereas on the right side there is a tendency in each branch to arise directly from the subclavian artery.

II. There are five important, and not infrequent, anomalies to which the attention is directed:

(a) The origin of the right subclavian artery from the descending part of the arch of the aorta. This occurs 4-6-8 times in 1000 cases (0.5% to 1% of all persons).

(b). Variableness in the origin of the transverse cervical artery, especially on the right side.

(c). The presence of a middle thyroid artery (*Thyroidea Ima*).

(d). The suprascapular artery arising from the internal mammary artery.

(e). The lateral thoracic artery arising from the internal mammary artery.

III. Eighty per cent of the dissections were made in negro subjects, a large number of whom may have been mulattoes or mixed bloods. That hybrids tend toward variation is a recognized biological law. This may explain the unusually large number of abnormalities encountered.

IV. Twenty-three infants were dissected and many of these show irregularities, particularly in the distribution of the suprascapular artery, which is frequently deficient, its place being taken by the dorsal scapular artery.

V. The branches of the subclavian artery may be more numerous in adults than in infants. The branches rise from all parts of the artery in adults, whereas in infants the branches frequently rise in a bunch from Part I.

TABLE 1.¹

THE ORIGIN OF THE ARTERIA MAMMARIA INTERNA (INTERNAL MAMMARY ARTERY).

Origin.	Quain.	Bean.
From Part I935	.806
From Part II003	.000
From Part III020	.000
From the trunus thyreo-cervicalis.....	.050	.097
From a common trunk with the A. trans. scap. and the A. trans. colli.....	.015	.007
From a common trunk with the A. transversa scapulae.....	.012	.000

TABLE 2.

SHOWING THE ARTERIES THAT COMPOSE THE TRUNCUS THYREO-CERVICALIS (THYROID AXIS) IN THE DIFFERENT TYPES.

Truncus thyreo-cervicalis composed of	Type I Fig. 2.	Type II Fig. 3.	Type III Fig. 4.	Type IV Fig. 5.	Not Classified.
A. thyroidea inferior	1	1	1	1	0
A. transversa colli	0	1	0	1	0
A. transversa scapulae	1	1	0	1	0
T. t. c. gives off A. mammaria interna..	0	0	0	0	1
Frequency of each type—Thomson.....	.417	.413	.060	.064	.044
Frequency of each type—Bean273	.257	.273	.120	.077

TABLE 3.

THE ORIGIN AND ANOMALIES OF THE A. THYROIDEA INFERIOR (INFERIOR THYROID ARTERY).

Origin.	Thomson.	Quain.	Bean.
As shown in Type I.....	.432296
As shown in Type II.....	.430	.900	.277
As shown in Type IV.....	.036	.	.129
From Part I (alone), as a single branch.....	.095	.101	.287
From the A. carotis communis001	.003	.000
From the A. thyroidea ima.....	.001	.011	.009
With the A. transversa colli.....	.038	.005	.065
With the A. mammaria interna046
With the A. vertebralis005	.001	.027
With the A. occipitalis018	.009
With the A. cervicalis superficialis.....	.053	.025	.074
With the A. cervicalis profunda.....027
 Anomalies.			
Absent022	.018
Very large—larger than the A. subclavia.....011	.027
Very small—not supplying the gland.....014	.065
One branch to the gland.....046
Two branches to the gland.....954
Forming both the A. occipitalis and the A. vertebralis.....009

¹ The figures in the tables are all given in percentages or may be considered as the number of times per thousand by removing the decimal point.

TABLE 4.

THE ORIGIN OF THE A. CERVICALIS ASCENDENS (ASCENDING CERVICAL ARTERY).

Origin.	Thomson.	Bean.
From the A. thyroidea inferior902	.656
From the truncus thyreo-cervicalis030	.099
From the A. transversa colli022	.091
From the A. cervicalis superficialis038	.077
From Part I004	.054
From the A. transversa scapulae002	.015

TABLE 5.

THE ORIGIN OF THE A. TRANSVERSA SCAPULÆ (SUPRASCAPULAR ARTERY).

Origin.	Thomson.	Quain.	Bean.
From Part I, with other arteries or alone776	.885	.813
From Part II, with other arteries or alone024	.015	.042
From Part III, with other arteries or alone074	.088	.101
From the A. axillaris015	.015	.042
From the A. subclavia alone (as a single trunk)057	.167	.219
With the A. transversa colli080	.080	.118
With the A. mammaea interna016	.020	.101
With the A. subscapularis005	.017
Double015025

TABLE 6.

THE ORIGIN OF THE A. TRANSVERSA COLLI (TRANSVERSE CERVICAL ARTERY).

Origin.	Thomson.	Quain.	Bean.
From Part I, with other arteries or alone508	.429	.365
From Part II, with other arteries or alone014	.191	.390
From Part III, with other arteries or alone477	.380	.245
With the A. transversa scapulae079	.048	.145
With the A. thyroidea inferior036072
With the A. thoracalis lateralis012
Double005012

TABLE 7.

THE ORIGIN OF THE RAMUS DESCENDENS (POSTERIOR SCAPULAR ARTERY).¹

Origin.	Deaver. ¹	Thomson.	Quain.	Bean.
From Part I003	...
From Part II005	.008	.150	...
From Part III023	.508	.353	.046
From the A. transversa colli968	.484	.480	.918
From the A. axillaris001009

TABLE 8.

THE ORIGIN OF RAMUS ASCENDENS (NOT GIVEN BY THOMSON OR QUAIN).

Origin.	Bean.
From Part I030
From Part II010
From Part III030
From the A. transversa colli730
From the truncus thyreo-cervicalis or its branches200

¹ Deaver, Anomalies of the Posterior Scapular Artery, University Medical Magazine, Phila., Vol. II, p. 151, 1889.

TABLE 9.

THE ORIGIN OF THE A. CERVICALIS SUPERFICIALIS (SUPERFICIAL CERVICAL ARTERY).

Origin.	Thomson.	Quain.	Bean.
From the A. transversa colli589	.963	.590
From the A. thyroidea inferior312	.004	.220
From the A. transversa scapulæ092	.026	.190
From the A. subclavia002	.008	...

TABLE 10.

THE ORIGIN OF THE A. INTERCOSTALIS SUPREMA (SUPERIOR INTERCOSTAL ARTERY).

Origin.	Quain. Right.	Quain. Left.	Bean. Right.	Bean. Left.
From Part I086	.220	.030	.075
From Part II482	.194	.105	...
From the truncus costo-cervicalis400	.370
From the A. vertebralis007		.020	
From the A. thyroidea inferior004		...	

TABLE 11.

THE ORIGIN OF THE A. CERVICALIS PROFUNDA (DEEP CERVICAL ARTERY).

Origin.	Quain.	Bean.
From the truncus costo-cervicalis820
From the A. intercostalis suprema932	...
From the A. subclavia049	.130
From the A. thyroidea inferior040
From the ramus descendens018	.010

The artery passes:

Above the first rib.....	.936	.820
Below the first rib.....	.064	.180

TABLE 12.

A COMPARISON OF THE MOST FREQUENT ORIGIN OF THE BRANCHES ON THE TWO SIDES OF THE BODY.

Artery.	RIGHT SIDE.		LEFT SIDE.	
	Origin.	%	Origin.	%
From :				
Vertebralis	Part I	97	Part I	88
Mammaria interna	Part I	95	Part I	82
Truncus thyreo-cervicalis	Part I (5 times)	11	Part I (25 times)	45
Thyroidea inferior	Part I	74	Truncus thyreo-cervicalis	45
Transversa scapulæ	Part I	54	Truncus thyreo-cervicalis	45
Transversa colli	Part II	54	Truncus thyreo-cervicalis	49
Ramus asc. A. tr. colli	Part II	66	Truncus thyreo-cervicalis	80
Ramus desc. A. tr. colli	A. tr. colli	97	A. transversa colli	90
Cervicalis superficialis	A. tr. colli	45	A. transversa colli	76
Cervicalis ascendens	A. thyroidea inferior	70	A. thyroidea inferior	70
Truncus costo-cervicalis	Part I	78	Part I	100
Intercostalis suprema	Tr. costo-cervicalis	86	Tr. costo-cervicalis	80
Cervicalis profunda	Tr. costo-cervicalis	82	Tr. costo-cervicalis	77

TABLE 13.

COMPARISON OF THE NUMBER OF BRANCHES FROM EACH PART OF THE SUBCLAVIAN ARTERY ON THE TWO SIDES OF THE BODY.

	Right.	Left.
Part I	80%	83%
Part II	11%	5%
Part III.....	8%	9%
A. axillaris	2%	3%

TABLE 14.

THE PERCENTAGE OF BRANCHES FROM PARTS II AND III OF THE SUBCLAVIAN ARTERY.

	Part II.		Part III.	
	Quain.	Bean.	Quain.	Bean.
None	19%	67%	54%	66%
One	67% ¹	24%	46% ¹	24%
Two	12%	8%	3%	4%
Three	9%	7%	8%	8%

¹ Quain counted a muscular twig which was not counted in this work.

TABLE 15.
ORIGIN OF THE BRANCHES OF THE SUBCLAVIAN ARTERY ON THE LEFT SIDE OF THE BODY.

TABLE 16.
ORIGIN OF THE BRANCHES OF THE SUBCLAVIAN ARTERY ON THE RIGHT SIDE OF THE BODY.

	No. arteries worked out.	A. cervicalis superficialis.	A. cervicalis superrificialis.	Absent.
A. vertebralis	66	69	64	5
A. mammaria interna	61	5	42	1
Truncus thyro-cervicalis	5	5	1	50
A. thyroidea inferior	45	5	35	1
A. transversa scapulae	32	8	44	1
A. transversa colli	4	12	21	1
Ramus ascendens A. trans. colli	3	2	3	6
Ramus descendens A. trans. colli	1	1	57	7
A. cervicalis superficialis	24	..
A. cervicalis ascendens	4	9	5	8
Truncus costo-cervicalis	45	10	1	2
A. intercostalis suprema	3	3	..	42
A. cervicalis profunda	5	..	1	45
Total	273	50	29	59
			121	88
			128	5
			49	1
			674	

In the above tables truncus thyro-cervicalis is considered to divide into three branches—A. thyroidea inferior, A. transversa colli, and A. transversa scapulae.

TABLE 17.
THE DISTRIBUTION OF THE BRANCHES OF THE SUBCLAVIAN ARTERY.

TABLE 18.

MUSCLES SUPPLIED BY THE SUBCLAVIAN ARTERY, WITH THE BRANCHES SUPPLYING THEM.

	<i>M. platysma:</i>
Almost constant.....	{ A. cervicalis superficialis. A. cervicalis ascendens.
Frequent.....	{ A. thyroidea inferior. A. transversa scapulæ. A. transversa colli.
Occasional.....	{ A. subclavia, Part I. Twigs. Truncus thyreo-cervicalis. Twigs.
	<i>M. trapezius:</i>
Constant.....	{ R. ascendens transversa colli. Two or more large branches passing between trapezius and rhomboids. R. descendens transversa colli. Several small branches. Some twigs after passing rhomboids. A. cervicalis superficialis. Several small branches.
Frequent.....	{ A. transversa scapulæ. One large branch just at bend to drop over scapula.
Occasional.....	{ A. cervicalis ascendens. Small branches. A. subclavia. Small branches.
	<i>M. rhomboids, and serratus posterior:</i>
Constant.....	{ R. descendens transversa colli. One large branch under rhomboids (anterior), and several smaller ones into the muscles.
Frequent.....	{ R. ascendens transversa colli from inferior branch between rhomboids and trapezius. A. cervicalis superficialis. Small branch or branches.
Occasional.....	{ A. cervicalis profunda. Small branch or branches. A. subclavia. Small branch or branches.
	<i>M. levator scapulæ:</i>
Constant.....	{ R. ascendens transversa colli. Small branches. R. descendens transversa colli. Small branches. A. cervicalis superficialis. Small branches.
Frequent.....	A. cervicalis ascendens. One large, several small branches.
Occasional.....	A. transversa scapulæ. Small branch.
Rare.....	A. cervicalis profunda. Small branch.
	<i>M. serratus anterior:</i>
Constant.....	R. descendens transversa colli. Small branches.
Frequent.....	R. ascendens transversa colli. Small branches.
Occasional.....	A. transversa scapulæ. Small branches.
	<i>M. supraspinatus:</i>
Constant.....	{ A. transversa scapulæ. One large branch. R. descendens transversa colli. Several small branches.
Occasional.....	A. circumflexa scapulæ. One large branch.

M. infraspinatus:

Constant.....	$\begin{cases} \text{A. transversa scapulae. One large branch.} \\ \text{R. descendens transversa colli. Several medium branches.} \end{cases}$
Frequent.....	A. circumflexa scapulae. One large branch.

M. latissimus dorsi:

Constant.....	$\begin{cases} \text{R. descendens transversa colli.} \\ \text{A. subscapularis.} \end{cases}$
---------------	--

M. subscapularis:

Constant.....	R. descendens transversa colli.
Frequent.....	A. transversa scapulae.

M. sternocleido-mastoideus:

Constant.....	$\begin{cases} \text{A. transversa scapulae. Small and medium branches.} \\ \text{A. cervicalis superficialis. Small and medium branches.} \\ \text{A. cervicalis ascendens. Small and medium branches.} \end{cases}$
Frequent.....	$\begin{cases} \text{Truncus thyro-cervicalis. Small and medium branches.} \\ \text{A. thyroidea inferior. Small and medium branches.} \end{cases}$
Rare.....	R. descendens transversa colli.

M. omohyoideus:

Constant.....	$\begin{cases} \text{A. cervicalis superficialis, or branches from.} \\ \text{A. transversa colli.} \end{cases}$
Frequent.....	A. transversa scapulae.
Occasional.....	$\begin{cases} \text{A. thyroidea inferior.} \\ \text{A. cervicalis ascendens.} \end{cases}$
Rare.....	A. subclavia. Three times.

M. sterno-hyoideus, and sterno-thyroideus:

Constant or frequent.....	A. thyroidea inferior.
Occasional.....	A. transversa scapulae.
Rare.....	A. subclavia.

M. scalenus anterior, medius, and posterior:

Constant.....	$\begin{cases} \text{A. cervicalis ascendens. Many branches.} \\ \text{Truncus costo-cervicalis, or its branches.} \\ \text{A. intercostalis suprema (R. dorsalis).} \\ \text{A. cervicalis profunda.} \end{cases}$
Frequent.....	$\begin{cases} \text{A. cervicalis superficialis, or branches from.} \\ \text{A. transversa colli.} \end{cases}$
Occasional.....	R. ascendens and descendens transversa colli.
Rare.....	A. subclavia.

M. longus colli:

Constant.....	$\begin{cases} \text{A. cervicalis ascendens.} \\ \text{Truncus costo-cervicalis, or its branches.} \\ \text{A. intercostalis suprema.} \\ \text{A. cervicalis profunda.} \end{cases}$
Frequent.....	A. thyroidea inferior.
Rare.....	A. subclavia.

M. longus capitis:

Constant..... A. cervicalis ascendens.

M. splenius capitis, and cervicis:

Constant.....	$\left\{ \begin{array}{l} A. \text{ cervicalis profunda. Through complexus } M. \\ A. \text{ princeps cervicis. Many small branches.} \end{array} \right.$
Frequent.....	$\left\{ \begin{array}{l} R. \text{ ascendens transversa colli. Several medium branches} \\ \text{between } M. \text{ splenii and trapezius.} \end{array} \right.$
Occasional.....	$\left\{ \begin{array}{l} A. \text{ cervicalis superficialis. Several medium branches be-} \\ \text{tween } M. \text{ splenii and trapezius.} \end{array} \right.$
Rare.....	$\left\{ \begin{array}{l} A. \text{ cervicalis ascendens. Branch through above fifth ver-} \\ \text{tebra takes the place of } A. \text{ cervicalis profunda.} \end{array} \right.$

M. semispinalis capitis and longissimus capitis (complexus, and trachelo mastoid):

Constant.....	$\left\{ \begin{array}{l} A. \text{ cervicalis profunda. Many medium branches beneath} \\ \text{the muscles.} \\ A. \text{ princeps cervicis. Many medium branches beneath the} \\ \text{muscles.} \\ A. \text{ vertebralis. Many medium branches beneath the} \\ \text{muscles.} \end{array} \right.$
Frequent.....	$\left\{ \begin{array}{l} R. \text{ ascendens transversa colli. Around edge of splenii, or} \\ \text{through them.} \end{array} \right.$
Occasional.....	$\left\{ \begin{array}{l} A. \text{ cervicalis superficialis. Around edge of splenii, or} \\ \text{through them.} \end{array} \right.$
Rare.....	$\left\{ \begin{array}{l} A. \text{ cervicalis ascendens. Branch through above fifth ver-} \\ \text{tebra takes the place of } A. \text{ cervicalis profunda.} \end{array} \right.$

M. semispinalis cervicis, and longissimus cervicis:

Constant.....	$\left\{ \begin{array}{l} A. \text{ cervicalis profunda. Small branches.} \\ A. \text{ intercostalis suprema (R. dorsalis).} \end{array} \right.$
Frequent.....	R. ascendens transversa colli. Small branches.
Occasional.....	$\left\{ \begin{array}{l} R. \text{ descendens transversa colli. Small branches.} \\ A. \text{ cervicalis superficialis. Small branches.} \\ A. \text{ cervicalis ascendens. Small branches.} \end{array} \right.$

M. spinalis cervicis, and iliocostalis cervicis:

Constant.....	A. intercostalis suprema (R. dorsalis).
Occasional.....	$\left\{ \begin{array}{l} R. \text{ descendens transversa colli. Small branches.} \\ A. \text{ cervicalis superficialis. Small branches.} \\ A. \text{ cervicalis ascendens. Small branches.} \end{array} \right.$

M. multifidus spinae, interspinæ, and intertransversæ:

Constant.....	$\left\{ \begin{array}{l} A. \text{ vertebralis.} \\ A. \text{ cervicalis profunda.} \end{array} \right.$
---------------	---

M. diaphragma:

A. mammaria interna.

M. rectus abdominis:

A. mammaria interna.

Shoulder joint:

- | | |
|---------------|--|
| Constant..... | { A. transversa scapulæ.
A. circumflexa scapulæ. |
| Frequent..... | { A. transversa colli.
A. cervicalis superficialis. |

Clavícula:

- A. transversa scapulæ.

Processus acromialis:

- A. transversa scapulæ.
A. cervicalis superficialis, or transversa colli.

Sternum:

- A. mammaria interna.
A. transversa scapulæ.

Glandula thyroidea:

- A. thyroidea inferior.

Glandula mammaria:

- A. mammaria interna.

ON THE OCCURRENCE OF SHEATH CELLS AND THE
NATURE OF THE AXONE SHEATHS IN THE CENTRAL
NERVOUS SYSTEM.

BY

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In a previous paper dealing with the development of the neuroglia tissue, a brief note was made of the occasional observance of certain half-moon or seal-ring cells encircling the medullated nerve fibers of the developing spinal cord of the foetal pig. It was stated that these cells appeared more numerous in the spinal cords of pigs between 16 and 25 centimeters, the period of most active medullation, and that in transverse sections they appear as seal-rings or crescents encircling the medullating axones and that they closely resemble the "nerve corpuscles" or "Schwann's corpuscles" which have been described clasping the medullated fibers of the peripheral nervous system. It was also suggested that, while in the light of recent investigations these cells have probably little or nothing to do with the formation of the myelin of the medullary sheath, they may have to do with the development of the supporting framework of that sheath.

Adamkiewicz, who first described the cells upon the fibers of the developing peripheral nerves, referred to them as "nerve-corpuscles," and by way of distinction I have referred to the similar cells observed upon the fibers of the central system as "seal-ring cells." This name only applies to their appearance in transverse section and is non-committal as to their particular function.

The purpose of this paper is to give a further description of these seal-ring cells based upon a more extended study of their occurrence and variations, and to offer a suggestion as to their relation to the medullary sheath. Some attention has necessarily been given to the nature of the supporting framework of the medullary sheath.

MATERIAL AND METHODS.

Pig material has been used almost exclusively in the observations in that the different ages could be easily obtained. All the study has been
AMERICAN JOURNAL OF ANATOMY.—VOL. IV.

made upon the spinal cord alone, and usually upon pieces taken from the cervical region. Preparations from the adult hog were compared with similar preparations from the adult human. After some study of younger stages, it appeared that only pigs of about 16 centimeters in length and above were necessarily concerned in the study of the structures involved. Prior to this all the fibers of the spinal cord are non-medullated or in the very early stages of medullation and none of the nuclei and their surrounding protoplasm show evidences of the differentiation in mind. The study, therefore, has chiefly involved foetal pigs of 16, 19, 21 and 28 centimeters, suckling pigs of about two weeks, and the adult.

Both transverse and longitudinal sections were used, supplemented by teased preparations. Some of the sections from each specimen were prepared by the Benda neuroglia method as employed by Huber. Others were made from pieces fixed either in Zenker's fluid or Van Gehuchten's mixture and stained lightly with haematoxylin and counterstained with congo red. The latter method was employed in that, with other tissues, congo red is efficient for bringing out cell outlines. Also sections from certain of the stages after medullation had begun and sections from the adult were stained by Mallory's method for white fibrous tissue. Other sections of the adult spinal cord were subjected to the action of pancreatin by the method of "digestion on the slide" described by Flint.

The pieces of spinal cord of different ages from which the teased preparations were made were first split with a sharp razor into thin longitudinal strips about one centimeter long and these were fixed in a mixture of equal parts of saturated aqueous corrosive sublimate and 1 per cent osmic acid. In order to fix the strips straight and extended, they were at first merely immersed in the fluid and then placed in adherence to the walls of the vials containing the fluid with the lower end of each strip alone in the fluid. Corking the vials and allowing the fluid to act for 10 or 15 minutes was found sufficient to stiffen the strips so that they would remain straight and extended when shaken down into the fluid. After doing this they were allowed to remain in the fluid for 12 to 24 hours. The strips were then washed for several hours in water frequently changed.

Especially in the younger stages the closely bundled nerve fibers of the spinal cord are so friable that it was found impossible to satisfactorily dissociate them with even the finest teasing needles. Even after dehydration and while in clearing oil (in which condition nerve fibers usually tease more easily on the slide than when in water and may be mounted in balsam immediately without disturbing their separated positions) but few pieces of isolated fibers could be obtained of sufficient length for satisfactory study. Teasing in glycerine with needles gave no better

results and glycerine mounts are very inconvenient for work with high power objectives.

After considerable trial, teasing with a fine stream of water was found to give much the best results with the material. The following simple arrangement proved very efficient: A strip of the fixed tissue was pinned by one end to the middle of a small piece of thin, carefully smoothed wooden board 4 or 5 inches long by 1 inch wide. With the free end of the specimen downward, the board was held in the left hand with its lower end resting at a slight angle upon the bottom of a stender dish of suitable dimensions, while with the right hand a stream of water the size of a fine needle was directed upon the specimen. The bits of dissociated tissue wash down into the stender dish, and a wooden board seems to give less spattering and rebounding of the water than a strip of glass, especially a glass slide with a cell or groove in it. A piece of 8-millimeter glass tubing drawn out to the required capillary dimensions and broken off squarely and the large end thickened and bound securely into a piece of rubber tubing was used in obtaining the sufficiently fine stream of water. This was securely attached either to the tap direct, and the tap water used, or attached to a Woulfe bottle containing distilled water under pressure, the pressure being obtained by attaching the reverse side of the Woulfe apparatus to an air force pump.

With sufficient pressure, a strip of tissue is washed down to a shred in a few minutes. The stream is best directed against it with a slight up and down, brushing motion. After two or three strips have been teased, or until the stender dish is nearly full of water, the dish is set aside and another taken if required. The nerve fibers being stained black by the osmic acid, the stender dish is placed upon a white surface. The material soon settles to the bottom of the stender and the water may be practically all withdrawn. Then the material from several stenders may be all transferred to one smaller stender and again allowed to settle. Then more water may be withdrawn and the material counterstained if desired.

For counterstaining I used 1 per cent aqueous acid fuchsin, which is known to stain osmic material readily and can be easily controlled. It was only desired in the teased preparations to demonstrate the shape and position of the sheath cells. After being in the fuchsin the required time, the material was washed first in 50 per cent and then in 70 per cent alcohol. It settles in alcohol more quickly than in water and the alcohol may be drawn off more completely.

Dehydration was completed with 95 per cent alcohol and finally with two changes of absolute. Then the absolute was replaced with a clearing

oil composed of equal parts of carbolic acid crystals, xylol and oil of bergamot. Xylol alone will clear the material, but it acts more slowly and dries too quickly on the slide where it is necessary to spread out the material before mounting.

To mount, the greater part of the clearing oil was removed from the material settled on the bottom of the dish, and then with the points of fine forceps or with a teasing needle, a sufficient mass of the dissociated fibers were lifted out and placed upon the slide and the surplus oil then drained off or taken up by holding a bit of blotting paper near the mass. A drop of balsam was then added and the fibers gently spread through it. The cover glass placed squarely down upon the mount tends to further spread the fibers out. Thus a preparation is obtained which is permanent and upon which the oil immersion may be used with convenience.

Examination of the preparations shows that teasing with the stream of water results in three advantages not obtained by teasing spinal cord by the ordinary methods: (1) Bits of nerve fiber are obtained of considerably greater length than could be obtained with needles and the fibers are nearly all isolated instead of in compact, broken clumps as often results from needles. (2) The preparation is cleaner. The fibers are washed out, leaving most of the plexuses of blood-vessels and the coarser masses of connective tissue behind in the shred remaining pinned to the wooden board. (3) The fibers themselves are clean. The neuroglia fibers and nuclei and the general protoplasmic syncytium otherwise surrounding and adhering to the fibers is washed off, especially from those having acquired a medullary sheath. This adds greatly to the value of the method for the purpose herein, in that almost every nucleus to be seen is adhering closely to a nerve fiber and usually can be considered to represent one of the sheath cells in question.

Of the stains employed upon the sections, the Bénda method proved best for the seal-ring cells. The toluidin blue of this method seems to especially differentiate these cells, staining their granular cytoplasm a deeper blue than the general syncytial protoplasm and rendering the cells more easily found than is the case after the more ordinary staining methods. Most of the syncytium is stained light brownish-red by the alizarin of the method, thus giving a background of good contrast. The nuclei and the adult neuroglia fibers when present are, of course, stained a deep blue.

In all the figures the camera lucida was used in outlining the drawings and the magnification in each was that obtained with ocular 4 and objective $\frac{1}{2}$ (oil immersion, Zeiss).

THE APPEARANCE OF THE MEDULLARY SHEATH IN THE SPINAL CORD AND THE OCCURRENCE OF THE SEAL-RING CELLS.

Most of the observations upon the origin, development and structure of the sheaths of the nerve fibers have been made upon the fibers of the peripheral nerves. This is perhaps due to the greater ease with which peripheral fibers may be studied. Satisfactory fixation is easily obtained with bits of peripheral nerves, their fibers are more easily isolated, being supported and separated by abundant connective tissue, and they have thicker and stronger supporting sheaths than the fibers of the central system. Indeed, the fibers of the central nervous system are described as having no primitive sheaths, or sheaths of Schwann, at all.

Notwithstanding various conflicting theories, the general concensus with regard to the origin of the nerve axones and their medullary sheaths may be summed up in the following:

1. All axones arise as outgrowths or processes of "nerve cells."¹
2. Developing axones become invested by special cells which give rise to the sheath of Schwann but probably have nothing to do with the formation of the myelin of the medullary sheaths.
3. In development, the axone precedes, secondarily the sheath of Schwann appears upon it, and lastly, or simultaneously with the beginning of the sheath of Schwann, the myelin sheath begins to appear.
4. No sheath of Schwann and therefore no sheath cells are described for the nerve fibers of the central nervous system. In the peripheral system the sheath cells and therefore the sheaths of Schwann are of mesodermal origin.
5. In the order in which they have been advanced, the theories of the origin of the myelin sheath are: (a) that the myelin is formed through the agency of the sheath cells by a process something like that by which fat is formed by the fat cells (Ranvier); (b) that the myelin is formed at the expense of the outermost portion of the nerve axone (Kölliker); (c) that the myelin is of exogenous origin, formed in the blood and distributed from the blood-vessels to the axones (Wlassak); (d) that the myelin arises as the result of influences exerted by the axone upon the surrounding stroma (Bardeen). By "stroma" Bardeen refers to an apparent fluid substance enclosed about the axone by the already formed sheath of Schwann. The theory of Ranvier (supported by Vignal and others) is invalidated by the statement that there are no sheaths of Schwann in the central nervous system, while there are medullary

¹ See Kölliker: Ueber die Entwicklung der Nervenfasern. Anat. Anz., July, 1904, page 7.

sheaths present, and further, by the conclusion of Gurwitch that for the peripheral nerve fibers the sheath of Schwann has nothing in common with the medullary sheath. It has also been shown by Kolster and Bardeen that the myelin may begin to appear about the axone before the sheath of Schwann is evident, though, as a rule, the axone of the peripheral fiber is enclosed by the sheath of Schwann before the formation of the myelin is apparent.

6. The medullary sheath is composed of at least two parts, first, the myelin (lecithin, etc.), and second, its supporting framework. As early as 1862 Mauthner roughly depicted this framework for certain giant fibers of the trout and the coarser portions of it were observed in 1876 by Ewald and Kühne, who gave it the name "*neurokeratin*," suggesting it to resemble horn in that they found it to resist the action of certain digestive ferments. Since then it has been studied in greater anatomical detail by various investigators, more recently by Wynn and Hatai, whose observations were also made upon peripheral nerve fibers.

The seal-ring cells which I have observed in the spinal cord of the pig are somewhat puzzling both as to their origin and their function. In the first place, they appear to have a period of maximum abundance. In the spinal cord of pigs of about 21 centimeters they are more easily found than at any other stage I have examined. In transverse sections stained by the Benda neuroglia method, I have seen as many as three evident in a single field of the oil immersion. While often a field contains none at all, it usually requires but little search to find one, though the nucleus, situated in the thicker side of the ring, may not always be contained in the section. In the older foetus they seem less abundant, and in the suckling pig it becomes difficult to find them in sections, while in sections of the adult spinal cord satisfactory examples of them are even more seldom found. Their protoplasm seems to have been used up and a free nucleus clasping the side of a nerve fiber may possibly be a neuroglia nucleus instead.

There are no direct indications of seal-ring cells prior to medullation. None have been observed upon fibers in the earliest stages of medullation. The conditions before the accumulation of myelin has begun are represented in Fig. 1, which is taken from the frayed end of a longitudinal section of the future white substance of the cord of a pig of 11 centimeters. The axones (*a*) appear as well-defined threads much smaller than in the older stages and separated by or imbedded in the general protoplasmic syneytium (*s*) which is the early form of the neuroglia tissue, no masses of which show differential blue staining by the Benda method, nor any definite outlines indicating individual cells. The

nuclei at this stage all appear nearly similar and are simply imbedded in the common protoplasm of the syncytium, the amount about a nucleus depending upon its position.

This study has not involved the spinal cord of the very young stages and, perhaps for that reason, by none of the stains I have employed does the growing axone, before medullation or at any stage in my preparations, appear as a "group of fibrils," or fibril bundles, as described by Bardeen for the peripheral nerves and by others cited by him. The axone of the young spinal cord, at least from 6 centimeters up, appears as a nearly homogeneous strand of even caliber which increases appreciably in size with the growth of the specimen. At best its structure shows nothing more than a fine, elongated reticulum, the threads of which become heavier and more evident as the fiber approaches maturity. Though Bardeen's observations were confined to the developing peripheral nerves, my failure to note the fibril-group form of the young axone must



FIG. 1. From longitudinal section of white substance of spinal cord of pig of 11 centimeters. Benda method. *a*—nerve axones; *s*—syncytium. $\times 550$.

be due to unsuitable methods, or to the fact that I have not examined the very young stages, for it is hardly probable that the axone in the central system is essentially different from the peripheral axone. Also Bardeen states that as early as 2 centimeters, most of the peripheral (intercostal) nerve fibers of the pig are covered with embryonic myelin. This indicates that myelination occurs very much earlier in the peripheral than in the central nervous system of pigs, for not till about 16 centimeters have I observed any appearances at all suggestive of the illustrations he gives as representing embryonic myelin.

Fig. 2 represents the frayed end of a clump of axones from the white substance of the spinal cord of a pig of 16 centimeters teased by the water method. This material was fixed in the corrosive sublimate and osmic acid mixture and the teased fragments stained with acid fuchsin. The appearance of the interaxone substance was verified from sections of the same stage stained by other methods. The teased preparation is

preferable in that the axones, uncut, may be followed a considerable distance and being often washed clean of the interaxonic syncytium, they may be studied more closely. It is seen that even in the pig of 16 centimeters, a stage when the medullation of the peripheral nerves is well advanced, most of the axones of the spinal cord show no signs of medullation, being but slender threads (*a*) of more or less even contour, with the substance of the syncytium (*s*) adhering to them.

The fibers indicated by *b* in Fig. 2 show the appearance of the first stages of the accumulation of myelin, or at least the first stages to be observed after the technic here employed. The myelin begins as small globules of various shapes and sizes adhering to the axone, giving it a beaded appearance. The globules are but very slightly blackened by the osmic acid at this stage and then upon their surface only, making them appear as small blisters which resist the action of the water in teasing. When washed clean of other adherent substance they may be observed minutely and there is no sign whatever of the presence of any other sheath. Between adjacent blisters and connecting them there is usually discerned a thin film on the axone but not always. Usually the globule appears adhering to one side of the axone rather than evenly surrounding it. This form of the first appearance of the myelin upon the axone is similar to that described by Vignal, Westphal, Wlassak, Kolster, Bardeen and others.

Those of the observers who take into consideration the sheath of Schwann of the peripheral fibers, give varying accounts of the time of the appearance of the myelin. After examining the great amount of literature upon the subject, it seems that the sheath of Schwann usually appears upon the peripheral axone before the myelin begins to be deposited, but often simultaneously with its appearance, and sometimes after the appearance of the myelin. The latter sequence indicates that the sheath of Schwann is not concerned in the origin of the myelin.

The fiber indicated by *c* in Fig. 2 is an example of the most advanced stages of myelination to be found in the spinal cord of pigs of 16 centimeters. It is the only fiber found after considerable search through the preparations of this stage which apparently possesses a sheath cell, though the protoplasm of this cell is not distinctly differentiated. There is positively nothing indicating such cells upon fibers of earlier stages of medullation. In sections of specimens of this age stained by the Benda method I have found no cells distinctly clasping the medullating axone, and showing the definite outline and differential staining of those found in the later stages, and especially in pigs of 21 centimeters. Occasionally a nucleus may be seen upon the side of an axone with protoplasm sur-

rounding it which is apparently more compact and which stains a deeper blue than the protoplasm of the general syncytium, but instead of having a definite outline the protoplasm seems to grade off into that of the syncytium. This condition is apparent in fiber *c* of Fig. 2. In general, the nuclei of this stage are merely imbedded in the syncytial protoplasm and show the various types of the neuroglia nuclei.

The appearance of definitely formed seal-ring cells is shown in Fig. 3. In this figure are represented two small areas from transverse sections of the spinal cord of a pig of 21 centimeters stained by the Benda neuroglia method. The cells (*c*) here show the form suggesting the name given them. Their finely granular protoplasm stains a decidedly deeper blue than that of the now more sparse protoplasm of the general syncyt-

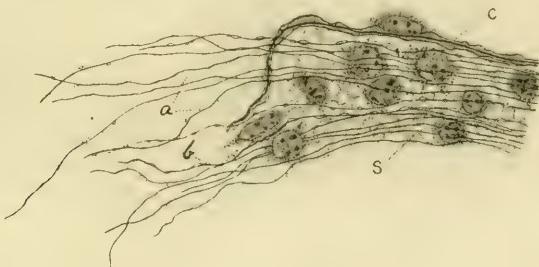


FIG. 2. From the spinal cord of a pig of 16 centimeters. Osmic acid and fuchsin. Teased by water. *a* = axones before medullation; *b* = axones showing beginning medullation; *c* = fiber in more advanced stage of medullation and with probable sheath cell; *s* = syncytial protoplasm. $\times 550$.

ium and their boundaries are definite. The fields were chosen because of each having two cells near together, three of the cells containing nuclei in the section. With the nucleus in the thicker side, the cells usually in this stage completely enclasp the fiber as a ring, but sometimes the protoplasm on the side away from the nucleus is either absent or so thin as to give the appearance of a crescent. Frequently a cell is found of the shape presented in *d*, where the protoplasm seems mostly extended from one side. In sections, the cells seldom seem to produce a depression in the medullary sheath for the fiber usually appears circular. Very probably none of the fibers possessing these cells are full grown, for the cells are found more abundant at about this age and the average diameter of the medullated axones here is much less than that of the adult. At

21 centimeters there are still many fibers in the spinal cord which have not acquired a myelin sheath (*a*). The syncytial protoplasm is less abundant probably because it is being transformed into neuroglia fibers (*n*) which begin to appear at this stage.

After birth cells of the seal-ring type are less numerous than in the pig foetus. Fig. 4, *c*, shows one as found in the suckling pig of two weeks. It is upon a larger fiber than those in Fig. 3 and the protoplasm of the cell is relatively less in amount and merely forms a crescent about the medullary sheath. The nucleus represented by *e* of this figure is probably a nucleus of a seal-ring cell which has no blue staining protoplasm about it. This is only inferred from its position, resting upon and here slightly indenting the medullary sheath. It may possibly be



FIG. 3.

FIG. 3. Areas from transverse sections of spinal cord of pig of 21 centimeters. Benda method. *c* and *d* = seal-ring cells; *a* = axones before medullation; *n* = beginning neuroglia fibers; *nn* = neuroglia nuclei. $\times 550$.



FIG. 4.

FIG. 4. From spinal cord of suckling pig of two weeks. Showing stage more advanced than Fig. 2. Otherwise same as Fig. 2. *c* = seal-ring cell; *e* = probably nucleus of former seal-ring cell (taken from a different field); *n* = neuroglia fiber; *nc* = neuroglia cell. $\times 550$.

one of the larger "free" neuroglia nuclei which has acquired this position. At this stage neuroglia nuclei are sometimes observed which have an area of more compact protoplasm about them and which stains darker than the, now scarce, granular protoplasm of the syncytium. One of these "neuroglia cells" is shown in the figure (*nc*) and such are described in many of the papers dealing with the neuroglia. Both this and the condition represented in the nucleus *e* are found in the adult material (see Fig. 7, *sc*).

In Fig. 5 are arranged some types of fibers selected from teased preparations of the spinal cord of the 21-centimeter pig fixed in the osmic acid mixture and counterstained with fuchsin. Three of these pieces of

fiber possess sheath cells and it will be noted that all three are fibers upon which the processes of medullation are well advanced. In the group indicated by *a* are three fibers not separated by the teasing and there is still present about them the syncytial protoplasm and some of the nuclei belonging to it (*s*). One of the axones of this group as yet shows no evidence of myelin, a condition which is quite frequent in pigs of this age. The fibers *b* and *c* were selected as showing the next stages in the acquirement of myelin. The medullated fiber with group *a* was included as illustrating the corrugated or ruffled outline of the growing sheath, an appearance frequently found and which suggests that it is an earlier stage than either of the fibers indicated by *e*, being a further develop-

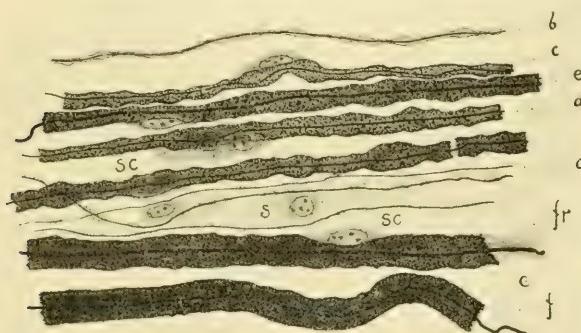


FIG. 5. Types of nerve fibers selected from teased preparations of spinal cord of a pig of 21 centimeters, showing stages of medullation and nature of seal-ring cells. Osmic acid and corrosive sublimate. Fuchsin. *s* = syncytium; *fr* = framework of sheath washed out in teasing; *sc* = seal-ring cells. $\times 550$.

ment of the smaller blistered or beaded form (*b* and *c*). The fiber *d* is perhaps in about the same stage, but it is doubtful whether either of the nuclei adhering to it represents sheath cells. The fiber *f*, showing an even contour of its sheath, is considered as illustrating the type of the most advanced stage in the growth of the medullary sheath found in pigs of 21 centimeters. This type is fairly numerous and often sheath cells are found upon it. The myelin of this type stains more darkly than that of the others, especially that of *b* and *c*, where it is much lighter and corresponds with certain of the descriptions of so-called "embryonic myelin."

In the late foetus and in the new-born (suckling pig of two weeks) the conditions more nearly resemble those of the adult. Medullation

has proceeded till there are much fewer fibers of types *b* and *c*, Fig. 5. At birth there may be found in the white substance, but very rarely indeed, fibers totally void of myelin such as one of those shown in group *a*. Sheath cells of the form of the seal-ring cells of the 21-centimeter pig are also more difficult to find in the later stages. This is apparently due to their being relatively less numerous and to the fact that when found they show relatively less protoplasm about their nuclei and about the fibers they clasp.

In the adult especially are unquestionable examples of these cells difficult to find. Usually the protoplasm has apparently been used up or so

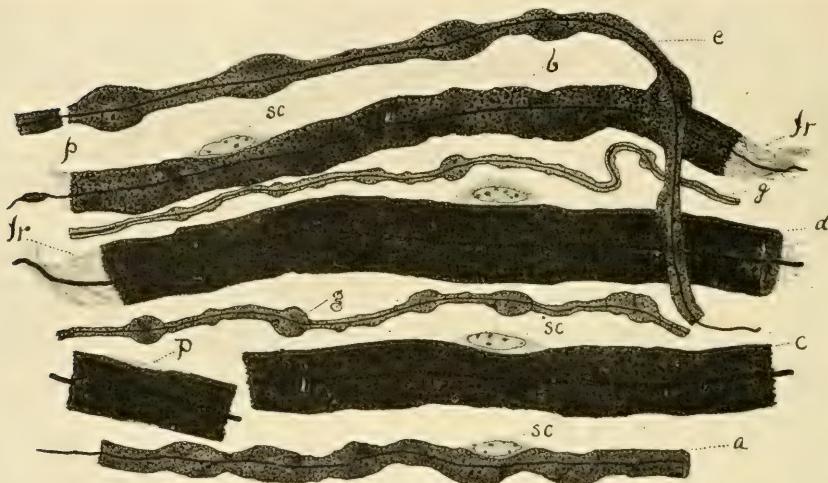


FIG. 6. Types of fibers selected from teased preparations of spinal cord of adult hog. Fixation, etc., same as in Fig. 4. *e* and *g* = types of small, thinly medullated fibers from white substance; *fr* = framework of sheath from which myelin has been washed in teasing; *sc* = sheath cells (sheath nuclei); *p* = peripheral sheath. $\times 550$.

dispersed that little more than the nucleus remains and the only suggestion that such nuclei do not belong to the general class of the neuroglia nuclei present throughout the inter-axone spaces, is their flattened shape and their position upon the nerve fibers, and, when observed in the teased preparations, the fact that they are not washed off in the process of teasing. It is possible, of course, that during growth neuroglia nuclei proper may be flattened against the medullary sheaths.

In Fig. 6 are presented some of the types of fibers to be found in the

spinal cord of the adult hog. They are all selected from teased preparations of the same specimen. Three of these pieces of fiber (*a*, *b* and *c*) possess what may be reasonably considered sheath cells. They were found after considerable search. Sometimes cells could be found adhering to the fibers in the manner similar to that shown upon the fiber *d*, but these were not considered as examples of the type sought. Cells situated in indentations of the sheath and with a more or less even outer contour were sought as more probable examples of the cell in mind.

There are many fibers in the adult cord relatively larger than the type *d*, but so far I have found no cells upon their sheaths, which latter are always deeply blackened by the osmic acid. Of the smallest medullated fibers in the adult cord, the types indicated by *e* and *g* are interesting. In the peculiar bulbous enlargements of their sheaths and in their relative size, they are identical with certain of the fibers described by Ranvier in the spinal cord of the dog. Ranvier pictured a nucleus with protoplasm about it adhering to what appears to be one of the larger of this type of fibers. So far I have not found examples of sheath cells upon any of them in the adult hog, but this may be due to the fact that such fibers are much less numerous in the cord than fibers of the larger type and that therefore a much less number of them was examined. Their peculiar appearance can hardly be considered wholly artifact, for the type is quite constant and often one of them may be followed unbroken for several millimeters and throughout shows the same form. It would be difficult to determine whether or not they are younger fibers in the process of medullation. They resemble certain of the Remak fibers. The few undoubted collateral branches I have seen in the preparations were of this general type of fiber.

The relation the sheath cells bear to the medullary sheaths of the central nervous system is as much of a question as it is in the peripheral nerves. When, in the study of this question, one examines into the nature of the medullary sheath in the spinal cord, it is immediately evident that it differs from that of the peripheral nerve fibers in several particulars.

While the fiber of the spinal cord is, of course, devoid of any structure similar to the capsule or sheath of Henle possessed by certain peripheral medullated fibers, it also lacks a distinct sheath of Schwann. Many deny that the nerve fiber of the central system possesses anything similar to the sheath of Schwann found on the peripheral fiber. Under certain conditions when the myelin is crushed or shrunken away, there may be seen occasionally evidences of a very delicate sheath about the periphery of the medullary sheath (*p*, Fig. 6). More usually, however, such must

adhere so closely to the myelin as to be invisible and to break with the breaking of the myelin sheath. This thin membrane-like appearance was first noted by Ranvier in the cord of the dog and he referred to it as a membrane. It has since been discussed by Schiefferdecker and others and its existence has been repeatedly denied.

The nerve corpuscles or Schwann's corpuscles of the peripheral nerve fibers are described as lying under the sheath of Schwann—between it and the myelin sheath. The cells here observed upon the fibers of the spinal cord seemingly lie upon the medullary sheath, being attached or in some way in close relation to it, without being enclosed upon it by a perceptible membrane. Sometimes in the teased preparations the protoplasm of the cells seems to blend into the blackened myelin (*e*, Fig. 5), but in the stained sections the protoplasm appears distinctly outlined from the myelin. The latter is perhaps the true condition, for whatever the function of the cells, the granular, more deeply staining portion probably only represents the untransformed endoplasm of the cell. In Fig. 7, *sc*, is shown one of the seal-ring cells as found in transverse sections of the spinal cord of the adult hog when stained by the Benda method. It is merely a nucleus practically free of endoplasm and its shape and position are the only features which suggest its being one of the cells in question.

I am as yet unable to reach a definite solution of the exact significance of these cells. If one follows them through the preparations with the idea that they are a distinct type of cell, having probably to do with the development of some part of the medullary sheath, the following observations may be made in support of this idea:

1. They do not appear differentiated till after the acquisition of myelin has begun.

2. When first indicated they do not appear as definitely outlined and differentiated cells, but rather their more deeply staining protoplasm seems to grade off into that of the general syncytium and to be continuous with it. This, and their being attached to a medullating fiber suggests that they are derived from nuclei and protoplasm formerly a part of the syncytium, and that their differentiation may be due to influences exerted upon it by the developing myelin.

3. During the period in which the process of myelination is at its height, they appear as distinctly differentiated cells with considerable protoplasm (or probably endoplasm) about their nuclei, often sufficient to completely encircle the growing sheath at the level of the nucleus.

4. With the further growth of the medullary sheath, the protoplasm or endoplasm of the cells is apparently used up gradually, till as the sheath

nears completion only the nucleus appears adhering to the periphery of the sheath. The fact that even such nuclei are rare in the adult suggests that they also may disappear.

5. In the relative abundance of their protoplasm at the different periods, these cells resemble the nerve corpuscles described for the peripheral nerve fibers and which are interpreted as having to do with the structures of the sheath.

ON THE FRAMEWORK OF THE MEDULLARY SHEATHS OF THE SPINAL CORD.

In none of my preparations of the spinal cord of the hog is there evident any arrangement in the medullary sheaths producing the appearance of the Schmidt-Lantermann clefts and segments described in the sheaths of the peripheral fibers. Especially is there no evidence of the heavy, separate interfitting cones described by Wynn. Hatai, who used a method much superior for the purpose to that used by Wynn, describes the structure of the peripheral medullary sheath as consisting of a network which contains the myelin. This "neurokeratin" network consists, he says, first, of two thin layers, one beneath the sheath of Schwann, and the other around the axone, the two being continuous at the nodes of Ranvier; and second, of a chain of cone-like formations, the bases of the cones being attached to the peripheral layer and the apices to the axone layer. Both the cones and the layers are highly reticular, exhibiting meshes of various sizes and shapes. He thinks the Schmidt-Lantermann clefts are produced artificially.

Hatai found formalin the best fixing agent in his study of this framework. Formalin is the fixing agent required in the Benda method and the alizarin used in the staining procedure of this method apparently brings out the framework of the medullary sheath in greater delicacy of detail than the stain used by Hatai. The toluidin blue of the Benda method stains the axone a dense blue as it does the developed neuroglia fibers, while the white fibrous connective tissue and the framework of the medullary sheaths is stained a light brownish-purple even to the finest fibrillæ. After formalin, as after many other fixing agents, the axone shrinks to considerable density and decrease in its normal diameter, but on the other hand, formalin seems to produce a slight swelling of the medullary sheath. In this process the framework of the sheath remains attached to the axone and is thus drawn or distended into a more open condition which renders the study of its detailed structure less difficult.

On comparing sections of peripheral nerve fibers with sections of the

white substance of the spinal cord, from both of which the myelin has been removed, it is evident at once that, whatever the detailed structure of the sheaths of the two, the framework of the peripheral medullary sheaths is somewhat stronger and heavier than that of the sheaths of the central fiber. This is true for human material and is especially true for the hog. The comparison may usually be made very readily with sections of the spinal cord, for nearly always portions of the ventral and dorsal roots have remained attached to the cord and, involved in the sections, are subjected to the identical technique in staining, etc., as the cord itself. In the peripheral nerve proper the framework of the medullary sheath is slightly heavier than in the nerve roots close to the pia mater. The nerve roots also do not possess the heavy connective tissue investments of the nerve outside the dura mater. Furthermore, in the hog the framework of the medullary sheath, both in the peripheral and central system, is apparently heavier than in man and the other vertebrates more usually studied. This is coincident with the well-known fact that in the hog the fibrous tissue framework of the organs, especially white fibrous and reticular tissue, is peculiarly abundant.

Under higher magnification the framework of the medullary sheaths of the hog shows a structure and arrangement capable of an interpretation somewhat different from that usually given. The structure certainly differs considerably from that pictured by Wynn. Wynn, however, used the Weigert staining method for medullated fibers and many of the appearances to be obtained by this method suggest that it is somewhat precipitative in its action upon the medullary sheath or that it may be classed among the impregnation methods. Its tendency certainly is to clog the finer structures rather than merely to dye them. Wlassak in his study of the origin of the myelin claims that the Weigert method stains only one substance of the medullary sheath. This substance he calls "*cerebrin*" and states that it is one of the constituents of the myelin. In this case, as Hatai points out, Wynn probably did not study the real framework of the sheath, the neurokeratin network of Hatai, but rather obtained pictures indicating the distribution of the cerebrin.

As stained by the Benda neuroglia method, the framework of the medullary sheath of the hog spinal cord appears as represented in Fig. 7. This figure contains fibers from both transverse and longitudinal sections of the cord. Each group was taken from an area near the periphery of the section or in the neighborhood of the pia, for the reason that near the periphery the framework appears heavier than toward the center and is always less collapsed and shrunken, due perhaps to better or earlier fixation near the surface of the specimen. It is seen that in transverse

sections stained by this method the framework supporting the myelin appears arranged in the form of concentric lamellæ. The different lamellæ, however, cannot be followed as distinct and individual membranes, for they apparently anastomose with each other and are further connected by still finer threads or branches. The structure is better described as a lamellated reticulum in the meshes of which the myelin is contained. Were it fibrillar in structure, lamellation could not appear in both transverse and longitudinal section. In longitudinal section the meshes of the reticulum appear considerably elongated in the direction of the long axis of the nerve fiber.

At the periphery of the fiber there is a slight condensation of this lamellated reticulum, giving under certain conditions the appearance of a membrane (*p.* Figs. 6 and 7), but close examination of the uncollapsed sections shows this membrane continuous with the more open network further in. This explains the difficulty with which the membrane is seen and the disputes concerning its existence, for consisting of but a condensed peripheral portion of the reticulated framework, the meshes of which are intimately occupied by the myelin throughout, the membrane is really continuous with the framework and therefore necessarily seems to adhere closely to the myelin. The breaking of the medullary sheaths in the fixed preparations consists, of course, of a breaking of the framework, and in the usual osmic acid and Weigert preparations especially, one could hardly expect to see the apparent membrane except in fortunate cases where the myelin is crushed or shrunken away from the periphery in such a way as to expose it. Quite frequently in the teased corrosive-osmic preparations the broken end of a fiber showed frayed portions of the framework of the sheath as indicated in Figs. 5 and 6, *fr.* These appearances are due to the myelin having been washed out of the meshes of a short extent of the framework by the action of the water in teasing and are probably not to be seen except in preparations teased by water.

The lamellated reticulum also usually shows a slight condensation about and upon the axone of the medullated fiber. This corresponds to the second thin layer of neurokeratin as described by Hatai. It probably corresponds to the *axolemma* frequently mentioned in the books. Being of the same nature and formed in the same way as the peripheral membrane, the usual difficulty with which it is seen is perhaps due to the same reason as that given to explain the difficulty with which the peripheral membrane is seen.

In the longitudinal sections of the fibers of the spinal cord it is seen that even the heavier lamellæ of the framework do not run uniformly

parallel with the contour of the fiber. At least after the manipulation in making the preparations, lines of adjacent lamellæ often appear collapsed upon each other, giving a resemblance of heavier lines running obliquely in the sheath. Often this collapse may be so great as to give openings in the framework, and sometimes, especially nearer the center of my sections of the cord, where fixation is perhaps less perfect, almost the whole framework may appear clotted against the axone or massed, axone and all, at one side of the section of the fiber. A partial collapse of this kind often occurs near the periphery also and gives rise to the

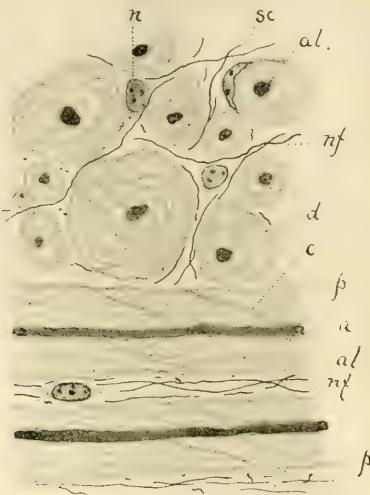


FIG. 7. Small areas from the outer portion of a transverse and of a longitudinal section of the spinal cord of an adult hog. Benda neuroglia method. *a* = axone; *al.* = axolemma; *p* = peripheral membrane; *n* = neuroglia nucleus; *nf* = neuroglia fibers; *sc* = seal-ring cell as seen in adult; *d* = sheath with collapsed framework. Detail of figure may be better observed with hand lens. $\times 550$.

appearance indicated in fiber *d*, Fig. 7. Again, in the less collapsed condition, one of the lines of collapse may appear in the longitudinal section to run slantingly from the axone to the periphery of the fiber (*c*, Fig. 7) suggesting one of the Schmidt-Lantermann clefts of the usual osmic preparations of peripheral fibers.

The framework of the medullary sheaths of the peripheral nerve fibers appears not only somewhat heavier than that of the fibers in the spinal cord, but also its arrangement is more varied and generally more complex. The form of the reticulated framework may be said to consist of

two general types with, however, all gradations between the two. In Fig. 8, *A* and *B*, there are represented examples of the extremes of the two types. They are chosen from among the dorsal root fibers of the adult specimen and show the nature of the framework as brought out by the Benda stain. The majority of the fibers in both the nerve roots show an arrangement of the framework conforming in various degrees to type *A*. The framework of type *B*, though somewhat heavier in structure, conforms quite closely with the general type found in the spinal cord. To illustrate type *B*, a piece of fiber involving a node of Ranvier (*nR*) was chosen to show the interesting fact that the lamellated reticulum of the sheath framework is not interrupted at the node. Not only are both

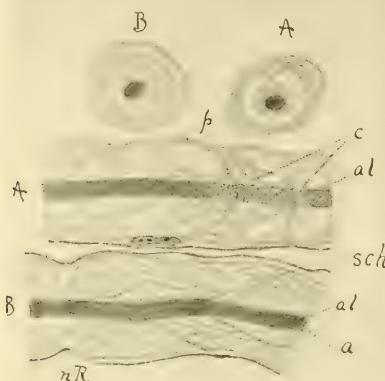


FIG. 8. Transverse and longitudinal sections of dorsal root (peripheral) nerve fibers from same preparations as Fig. 7. Showing extremes (*A* and *B*) of the two general types of framework of medullary sheaths. *sch* = sheath of Schwann; *n* = nerve corpuscle; *nR* = node of Ranvier; *c* = cone arrangements of framework; other letters = same as in Fig. 7. Use hand lens to observe details cited. $\times 550$.

the outer and inner "membranes" continuous through the node, as pointed out by Hatai, but also some of the intermediate lamellæ pass from one internode to the other. From the fact that but slight condensation is apparent at the nodal constriction, it is probable that the framework is less abundant at that point. Close examination shows that the reticulum suddenly narrows down by both a diminution in the number of its lamellæ and a diminution in the size of its meshes. The same general behavior is also apparent at the nodes in fibers of type *A*.

Sheath frameworks of the type *B* seem less frequent in the peripheral nerves proper than in the nerve roots within the dura mater. So far, however, I have examined sections of only one piece of peripheral nerve

stained by the Benda method. The root fibers were chosen for the illustrations because, being on the same slides as the sections of the spinal cord, they were subjected to the identical fixation, treatment and decolorization as the fibers in the cord, and were therefore deemed better for comparison with the conditions found in the cord.

The form of sheath framework shown in *A*, Fig. 8, is no doubt the form to which attention has been usually given in the literature. In this the reticulated framework is more or less condensed into interfitting conical partitions between masses of less intimately supported myelin. In this condensation the outer and inner "membranes" (*p* and *al*) are maintained and rendered even more evident. The partitions when in the form of cones are so arranged that the bases of the cones are continuous with the peripheral membrane and the apices with the axolemma. This is necessarily the case. Otherwise they would not be conical. When a sheath shows the decided conical arrangement for any considerable distance, which it very seldom does, the cones are not necessarily arranged parallel and interfitting throughout the distance. For a short extent they may be parallel, then irregular cones may be interposed or cones with their apices pointing in opposite directions. The most perfectly formed cones themselves contain openings of the same optical properties as the spaces between the cones. As stained here the cones appear to consist of a fibrillated reticulum with the meshes greatly elongated in the direction occupied by the cone between the axone and the periphery. In *A*, a fiber was found through which the knife passed obliquely at a region of more or less perfectly formed cones. This allows a certain amount of perspective. Luckily the region also possessed a sheath nucleus. It is here especially evident that the cones do not fit cleanly upon the axone but are continuous with the axolemma both above and below by a more irregularly dispersed portion of the reticulum in such a way as to give an appearance resembling an open umbrella, the rib-braces of which often extend to the apex of the adjacent cone (*c*, Fig. 8). The appearance shown in the transverse section indicated by *A* is frequently seen in the sections of peripheral fibers and is interpreted as a section involving the base of one cone and the apex of another. The heavier fibrillæ show a peculiar whirled arrangement which is probably due to the lamellæ of both cones being cut at levels where they are curving upon the axone in the one case and the periphery in the other.

Though frequently cones are to be found in the hog material somewhat longer than the example chosen in *A*, I have as yet seen none as long as certain of the cones pictured by Hatai and Wynn for the peripheral nerves of the animals they studied. The finely fibrillated reticular nature of

the framework suggests that the heavy cones pictured by Wynn may after all refer to the arrangement pictured here. His pictures probably represent the conical arrangement of the reticulated framework, the fine meshes of which had been clogged by the precipitate of the Weigert method.

The Schmidt-Lantermann clefts seen in the usual osmic preparations of the peripheral nerves are interpreted as representing the cones in longitudinal section. The cones, being condensed portions of the framework, which for that reason contain less myelin than other portions of the sheath, are therefore less blackened by the osmic acid. Light passing through them more readily results in the familiar appearances.

As is well known, in the ordinarily fixed material, every peripheral fiber does not show the clefts, and when they are shown they seldom appear in straight, even course slanting from the axone to the periphery. Further, the clefts never appear parallel along a considerable extent of a fiber, indicating that the cones do not all lie in the same direction. Again, it is invariably claimed that the clefts are not present in the fresh condition of the medullary sheath; that after death the sheath soon undergoes changes resulting in their appearance.

All the statements concerning cones and clefts in the medullary sheaths have been made with reference to the peripheral nerve fibers alone. I have found neither in the fibers of the spinal cord. In the peripheral fibers it seems to me that a partial explanation of the cones is suggested in the many varieties of arrangement of the framework of sheath to be found in the fixed and stained material. Considering types *A* and *B* of Fig. 8 as the two extremes, a study and arrangement in series of the intermediate forms may be made which will suggest that the intermediate forms are gradations from type *B* into type *A*; in other words, that the form of framework shown in type *B*, though less frequent in the preparations, may represent more nearly the normal arrangement and that type *A* is probably derived from type *B* through a procession of post-mortem changes. In the intermediate forms may be noted: (1) those in which the lamellated reticulum of type *B*, arranged more or less parallel with the axone, contains occasional small, oval or globular spaces interrupting the parallel arrangement; (2) those in which the spaces are more numerous, some of them much larger than others and so shaped and situated with reference to each other as to suggest that the larger spaces arise from a coalescence of the smaller; (3) those sheaths in which the larger spaces predominate, giving the framework a marked blistered or honeycombed appearance with the smaller spaces in the partitions between the larger; and finally, those which conform in

general to type A of Fig. 8. Here a continued coalescence of the spaces has resulted in some so large that they more or less completely encircle the axone and are bounded by necessarily condensed portions of the reticulated framework which, at the periphery of the sheath and about the axone, amount to little more than the outer and inner layers of Hatai. The partitions between adjacent large spaces themselves often contain numerous smaller spaces. These larger spaces encircling the axone may be often so shaped and arranged that in longitudinal section, either optically or by the microtome, the resulting partitions of the framework between them may easily appear in the more or less conical form. In the left-hand end of the bit of fiber indicated by A, the cones are less apparent than further to the right. Many of the fibers with numerous larger spaces do not show the conical arrangement of the framework, even as distinctly as is indicated in A.

The spaces are interpreted as occupied by globules of myelin, the larger resulting from a coalescence or fusion of the smaller globules. In the normal fresh condition the myelin is probably not in the form of globules at all but as a more finely divided emulsion evenly distributed throughout the framework supporting it. Globulation, beginning as very small globules which later coalesce into the larger, apparently results in a distortion of the natural arrangement of the framework of the myelin sheath. The beginnings of such may be noted even in B of Fig. 8 and in all fibers of its type. In accordance with this view the conical arrangement of the framework and all the intermediate forms may be looked upon as artifacts. The material of my preparations was taken at the slaughter house shortly after death and immediately placed into the fixing fluid. That the peripheral nerves were more exposed to the atmosphere and to handling probably explains why globulation is so marked in their fibers and not in those of the spinal cord. However, the material of my Brenda preparations of the human cord was taken 48 hours after death and in this also the sheaths of the central fibers do not show the conical arrangement of the framework. But it also was placed into the fixing fluid immediately upon removal from the body.

That the medullated sheaths of the peripheral nerve fibers after fixation in weak solutions of osmic acid or after poor osmic fixation usually present the appearance of a coarse network, is a matter of frequent observation. Lantermann himself noted such an appearance. Ewald and Kühne named it *neurokeratin*. The appearance is considered due to the myelin being in the form of imperfectly blackened globules, the interstices between the globules giving a merely optical impression of a network. Pertik, however, interpreted it as indicating the presence

of a substance between the globules which colored differently by the osmic acid. Boveri and Kupffer thought the appearance of the network to result from a stage of the decomposition of the myelin, while on the other hand, Gedoelst affirmed the preformation and preexistence of the network, agreeing with Pertik that it indicated the presence of two substances in the myelin sheath. More recently (1904) Chiò, experimenting with different solutions of osmic acid upon the peripheral fibers of the frog and guinea-pig, reached the conclusion that the globules are a constant form of the myelin. He finds globules present not only after the action of the osmic acid but after various other reagents as well, both isotonic and anisotonic. His illustrations, all of them after the action of osmic acid, present the appearances usually found in imperfectly blackened medullated fibers. By removing the blackened portions, the globules of myelin, from his pictures, all of them may be homologized with the form here indicated by *A* in Fig. 8 and with the gradation forms between *A* and type *B*.

Without doubt myelin exists normally in a finely divided form—an emulsion. For this reason the nerves possessing it appear white by reflected light. In the fresh condition, however, the individual droplets are much smaller than after post-mortem exposure and treatment with reagents. The larger globules, arising after death by a continual coalescence of the smaller, perhaps bear a similar relation to the condition in the fresh nerve as do the globules of cream bear to those of fresh milk. In the finely divided condition the myelin is distributed evenly throughout the sheath framework supporting it; in the coarser globular form the normal arrangement of the framework is distorted in the characteristic manner by the continued coalescence of the smaller into the larger globules, the conical appearances resulting from the necessary shape of the large globules within the confines of the cylindrical sheath.

In the central system there is no sheath of Schwann conforming to the distinctly formed membrane investing the fibers of the peripheral system. In my sections the sheath of Schwann of the peripheral fibers is well differentiated by the Benda stain. It seems everywhere to be completely separate and distinct from the framework of the sheath, as is shown in Fig. 8, *sch.* The sheath nuclei, usually pictured as adhering to the sheath of Schwann, by no means necessarily do so. Usually surrounded by a small amount of protoplasm, they may as often be found adhering to the surface of the medullary sheath as to the inner surface of the sheath of Schwann. The sheath of Schwann closely investing the medullary sheath, the nuclei are usually in contact with both.

The sheath of Schwann in both structure and staining properties resembles the ordinary basement membranes of the epithelia of the body.

Basement membranes are of connective tissue origin and are not cellular. The sheath nuclei may represent certain of the cells having to do with the development of the sheath of Schwann which were enclosed within the sheath and therefore separated from the similar nuclei distributed in the endoneurium outside the sheath. It is well known that, while an internode of the medullated peripheral fiber usually has a sheath nucleus, one is not present in every case, and further, a single internode may sometimes show two or more sheath nuclei.

On the other hand the sheath nuclei may have to do with the development of the framework of the myelin sheath as well as with the sheath of Schwann. The framework, both of the central and peripheral fibers, stains like the sheath of Schwann. It is suggested that the seal-ring cells of the foetus and the sheath nuclei of the adult spinal cord represent "cells" derived from the syncytium and whose activities result in the reticulated framework of the medullary sheath; that, wherever found, the protoplasm about the nucleus represents only the endoplasm which is being transformed into exoplasm, which in its turn is transformed into the lamellated reticulum by a process similar to that described by Mall in the development of the fibrous connective tissues; and finally, that the origin of the framework and the origin of the sheath of Schwann of the peripheral fibers may be similar.

The framework of the medullary sheath in the spinal cord resists the digestive action of pancreatin as first noted by Ewald and Kühne for that of the peripheral fiber. However, in the digested sections it does not appear so abundant as after the Benda neuroglia stain. After fixation in other fluids it does not appear as abundant as it does after fixation with formalin and also it stains very lightly or not at all by the ordinary staining methods. Mallory's method for white fibrous connective tissue stains it but lightly. The digested sections of the spinal cord stained by this method or with strong fuchsin solutions show more or less collapsed circles representing the transversely cut nerve fibers. These circles contain remnants of the framework usually so collapsed and washed together that little semblance of the original arrangement can be made out. Occasionally there is a small inner ring showing the opening from which the axone has been digested and representing the inner layer of the framework or axolemma with certain other portions of the framework collapsed upon it. The outer ring, representing the periphery of the fiber, is better maintained, due probably to the presence of the interstitial framework of the white fibrous connective tissue of the spinal cord.

SUMMARY.

1. There are present upon the medullated fibers of the spinal cord cells similar to the nerve corpuscles or sheath cells of the peripheral nerve fibers.
2. These cells are more abundant and possess more protoplasm during the period of the most active formation of the myelin sheath than during the later stages.
3. They do not appear upon the fibers till after the fibers have begun to acquire myelin.
4. They are apparently derived from the nuclei and protoplasm of the syncytium of the developing spinal cord and are perhaps differentiated through some influence exerted upon the syncytium by the developing myelin upon the axones.
5. These cells occur much more rarely upon the adult fibers and when found possess little or no protoplasm.
6. The framework of the medullary sheaths of the spinal cord occurs in the form of a lamellated reticulum in the meshes of which the myelin is supported.
7. This framework differs from that of the medullary sheaths of the peripheral nerve fibers in that it is not quite so heavy and always shows an arrangement parallel with the axone of the fiber.
8. The more or less parallel arrangement of the reticulum is probably the normal condition of the framework in the peripheral nerves also, the post-mortem appearance of the usually described coarse "neurokeratin network" being but a distortion of the normal arrangement produced by a continued coalescence of the much finer globules of the original myelin emulsion, the occasional conical arrangement of the framework representing the final result of the further coalescence of the globules.
9. The framework of the medullary sheaths of the spinal cord resists digestion as does that of the medullary sheaths of the peripheral nerves.
10. While there is a supporting contingent of white fibrous tissue among the nerve fibers of the spinal cord, the statement is confirmed that there is no distinct, separate membrane investing the fibers of the central system corresponding to the sheath of Schwann investing the medullated fibers of the peripheral nerves.
11. The sheath cells of the spinal cord are probably concerned in the development of the framework of the medullary sheath and probably in a manner similar to that in which the other fibrous supporting tissues of the body are developed.

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THE DEVELOPMENT OF THE LYMPHATIC NODES IN THE PIG AND THEIR RELATION TO THE LYMPH HEARTS.

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WITH 17 TEXT FIGURES.

Notwithstanding the numerous investigations on the lymphatic nodes, there are many points in regard to their structure and development in which our knowledge is not yet clear.¹ For example, such a fundamental question as the relation of the nodes to the lymphatic system as a whole and to the vascular system, in other words, the problem of general morphology: or, more special questions in regard to the nodes themselves; primarily the existence of a structural unit; and secondarily the relations of the endothelium of the channels to the reticulum within the node, whether the channels are open or closed, and the origin of the lymphocytes.

In regard to the problem of general morphology, we have previously shown² that the lymphatics are modified veins. They develop as blood-vessels do, by the budding of endothelial cells, and the direction of their growth is determined by the arteries and veins. In the lymphatic system there develop four lymph hearts which pulsate in the amphibia; but in the mammalian forms, at least in pig and human embryos, have no muscle in their walls. These lymph hearts drain the body, that is to say all the lymph passes through them before entering the veins. It will be shown in the present paper that the first lymph nodes in the body develop from the lymph hearts. That is, the organ which was a pulsat-

¹ During the past eleven years, there have been four extensive researches on the development of lymph nodes by Gulland, Sixer, Retterer, and Kling. Each of these authors has reviewed the literature. Gulland, Journal of Pathology and Bacteriology, Vol. 2, 1894; Sixer, Anatomische Hefte, Bd. VI, 1896 Retterer, Journal de L'Anatomie et de La Physiologie, 1901 Kling, Upsala Läkareförenings Förhandlingar, 1903, and Archiv f. mik. Anat. u. Entwicklungsgeschichte, Bd. 63, 1904.

² Sabin: American Journal of Anatomy, Vol. I, 1902.

ing heart in the amphibia becomes transformed into a node in the higher forms. The lymph heart is the point from which ducts radiate in development to drain a large area, generally speaking a quarter of the surface of the body, and here the primary lymph node develops. Subsequently there are secondary points, from which ducts growing from the lymph heart radiate to drain lesser areas, and here other nodes are formed. These are subcenters through which lymph passes before reaching the primary node.

As to the nodes themselves, the study of their development brings out that they are made of two fundamental structural elements. First, a lymphoid or adenoid tissue, consisting of lymphocytes in a reticulum around the blood-vessels making the lymph cords and germ centers; and secondly a lymphatic tissue, or sinus, made of large numbers of lymph ducts closely packed together. In brief, an ordinary lymph node is a blood vascular organ, made in part of structures derived from the blood-vessels and in part of structures derived from the lymphatics. The vascular unit of the node is the terminal artery and its capillary plexus, the artery being bordered by the cord and the capillary tuft surrounded by the germ center. These two elements, the vascular and the lymphatic, are found in varying proportions in the ordinary node. However, both in the embryo and in the adult, either element may be found alone. In the embryo and probably in the adult pig, there are small lymph follicles consisting of a tuft of blood capillaries surrounded by lymphocytes and entirely without a sinus. In the haemolymph node and in the spleen the same type of lymphoid tissue is found, but here the sinuses are made, not of lymph ducts, but of veins. Thus in the blood vascular organs the lymphoid element consisting of lymphocytes in the adventitia of an artery is constant, while the sinus element varies, being absent, or made of veins, or of the modified veins which are called lymphatics.

Throughout the paper certain terms have been adopted. Lymph node is used as a general name to cover all lymphatic glands; the term follicle is used to represent a simple node consisting of the structures that go with a single artery. The simplest node consists of one follicle; other nodes are groups of follicles. The follicle is the anatomical or structural unit; it is also the vascular unit. The follicle may be without a sinus, or surrounded by a lymphatic sinus or surrounded by a venous sinus.

The term lymph heart has been retained notwithstanding that in the pig there is no muscle in its wall at any stage. It is, however, a sac from which all the ducts for the skin radiate and in that sense is homologous with the lymph heart of the amphibia.

Material and methods.—The material for the present study has been embryo pigs of all stages. In studying the development of the nodes, just as in studying the development of the duets, it is essential to employ injections, both lymphatic and arterial, and these injections have been made in every stage. The lymphatics have been injected by means of a hypodermic syringe, with either saturated aqueous Prussian blue or with India ink. The material has been preserved for the most part by the injection of a saturated solution of bichloride of mercury, either into the aorta or into the umbilical vein. The blood-vessels are usually first washed out with warm salt solution, then the bichloride introduced and continued until the embryo is hard and white. The injection is made slowly with a pressure of about 100 mm. of mercury and the bichloride allowed to stay in the vessels from one-half to two hours. It is then washed out thoroughly by injecting 70 per cent alcohol through the same canula. The embryo is then placed in 80 per cent alcohol over night and the next day transferred to 95 per cent alcohol. This method involves the least possible shrinkage, indeed it may be made to produce a slight distension of the tissues, which is an especial aid in studying lymphatic nodes.³

In studying the developing nodes in fresh tissue, it is readily noticed that they are sometimes found distended with fluid and sometimes collapsed. It is just as easy to tell with the unaided eye when a node is thus distended with lymph as to distinguish between the mesenteric lymph nodes distended with chyle or collapsed and empty. This method of injection produces the same distension of the spaces that occurs normally when the node is in active function; that is to say, it makes the lymphatic duets rounded rather than collapsed. This explains the especial value of the method as applied to lymphatic tissue.

A valuable aid in the localization of the nodes, and especially in studying the relations of the lymph hearts to the developing nodes, is found by making injected embryos transparent.⁴ The lymphatics are first injected with India ink and then the entire embryo is placed in 95 per cent alcohol. They are left in the alcohol until they are shrivelled. This takes at least two weeks. The embryos are then cleared in a dilute solution of potash from 1 to 2 per cent, taking from 1 to 4 hours. The specimens are preserved in glycerine, at first 20 per cent and later in pure glycerine.

³ McFarland: *Jour. of App. Microscopy*, Vol. II, No. 10, and Myers, *Ibid.*, Vol. VI, No. 12, and J. H. Bull., 1905.

⁴ Mall: *American Journal of Anatomy*, Vol. IV, 1905, p. 6.

The lymph heart.—The present paper is a continuation of two papers previously presented in this journal, the first in Vol. I, 1901, and the second in Vol. III, 1904. It has been shown⁵ that the lymphatics bud off from the veins at the root of the neck, grow along the internal jugular vein on either side, and expand into a sac in the neck. This sac or lymph heart is shown in Fig. 1 as it appears in the neck of a pig 2.7 cm. long.

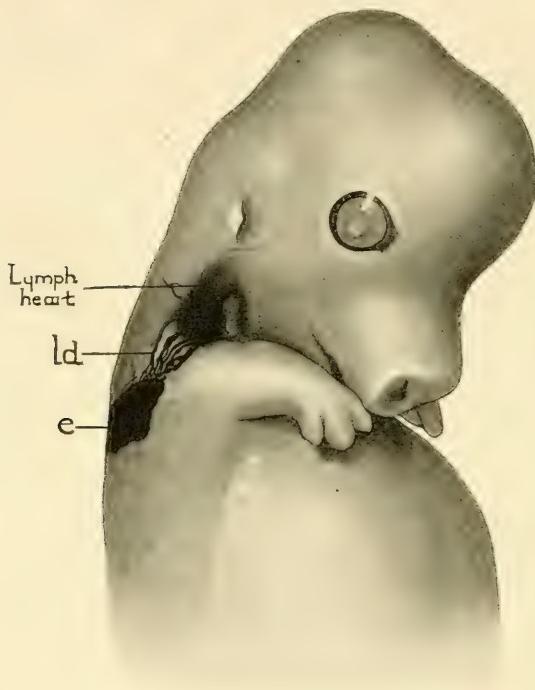


FIG. 1. Embryo pig, 2.7 cm. long, showing the anterior lymph heart injected. \times about $4\frac{1}{2}$. E, extravasation at the point of injection; ld, lymph ducts leading from the extravasation to the lymph heart.

The lymphatics were injected with India ink by a hypodermic needle introduced into the ducts over the shoulder at the point marked by the extravasation in the figure. The relation of this sac to the veins is shown in the accompanying diagram, Fig. 2. One duct of the sac lies along the course of the internal jugular vein. Just below the ear, under cover of the sterno-cleido-mastoid muscle, this duct widens into a sac which makes an arch in the neck. The sac curves outward and down-

⁵ Ibid., Vol. I.

ward with the apex, marked *a* in the diagram, near the surface, adjacent to a superficial vein over the shoulder. This apex is to be found in the triangle between the sterno-cleido-mastoid muscle and the trapezius. From the apex of the sac, a duct follows along the vein of the shoulder and empties into the duct along the internal jugular vein. This duct becomes the main duct of the sac.

Figure 3 is a section of the base of the sac at about the level marked *b* in Fig. 2. The section is cut transversely through the neck of a pig 2 cm. long and passes through the larynx. It shows the relation of the lymph heart both to the internal jugular vein and to the sterno-cleido-mastoid muscle. The lining of the sac consists of a single layer of endothelium.

Fig. 1, which shows the lymph heart and its ducts in a pig 2.7 cm. long, is to be compared with Figs. 1 and 2 in this journal, Vol. III, 1904, pp. 184 and 185, which show the superficial lymphatics in pigs 2.5 cm. and 3 cm. long.

From these three figures it can be seen that the lymph ducts or capillaries grow from the apex of the sac first to the skin of the shoulder and back of the head. Then ducts grow forward from the apex of the sac over the surface of the sterno-cleido-mastoid muscle, and form a long plexus around the external jugular vein parallel to the anterior border of the muscle. This plexus is shown in a little later stage in Fig. 6, and in section in Fig. 5. From this long plexus ducts first grow to the face as is shown in Fig. 2, Vol. III, p. 185. Later on it will be shown that at this stage, namely, when the pig is 3 cm. long, the apex of the sac begins to be transformed into a lymph node.

The condition of the lymphatic system in the neck of a pig 3 cm. long is as follows: There is first the lymph heart with its efferent ducts connected with the veins; then ducts have grown from the apex of the sac first to the skin of the shoulder and back of the head, and secondly to the face. The sac shows also the first rudiments of a lymph gland.

The maximum size of the lymph heart is attained when the pig is 3.6 cm. long, and in Fig. 4 is given a flat reconstruction of the sac at this stage. It was made from a set of serial sections and gives the size more accurately than the potash specimens. Fig. 5 is a section from the

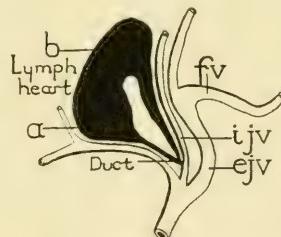


FIG. 2. Diagram showing the relation of the lymph heart, of Fig. 1, to the veins. The lymph heart is in solid black. *a*, apex of the lymph heart; *b*, base of the lymph heart; *ejv*, external jugular vein; *fv*, facial vein; *ijv*, internal jugular vein.

same series taken at the level marked *b* on Fig. 4. It corresponds with Fig. 3, and shows a similar relation to the internal jugular vein and the sterno-cleido-mastoid muscle. It shows that the sac comes nearest the surface between that muscle and the trapezius. It also shows the external jugular vein at the anterior border of the sterno-cleido-mastoid muscle and the neighboring plexus of lymph ducts. A section about half way between the letters *a* and *b* on Fig. 4 shows the heart near the surface and the duct adjacent to the vein as two distinct cavities.

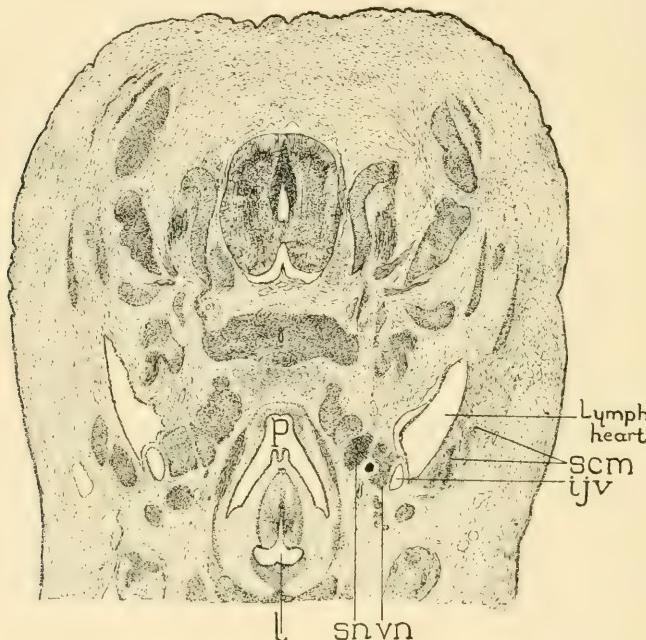


FIG. 3. Transverse section through the neck of an embryo pig, 2 cm. long, showing the anterior lymph hearts. $\times 15$. *I.Jv.*, internal jugular vein; *l.*, larynx; *p.*, pharynx; *scm.*, sterno-cleido-mastoid muscle; *sn*, sympathetic nerve; *vn*, vagus nerve.

Fig. 6 is another specimen made transparent in potash. It is from a pig 6 cm. long and is to be compared with Fig. 5, Vol. III, p. 188, which is from a pig 5.5 cm. long. The former shows the lymphatics in the depth and the latter those of the surface at about the same stage. The injection for this specimen was made in two places: one just back of the fore leg as marked by the extravasation; from this injection the ducts over the shoulder, the lymph sac, and two large ducts running to the long plexus were filled. The second injection was made between the eye

and ear, by which the capillaries over the face and the long plexus in the neck were filled.

In Fig. 6 the lymph heart is still plain, showing as a triangle in the depth. It has modified somewhat in shape, inasmuch as lymph nodes are forming at the apex and base, making the angles of the triangle appear as knobs. The apex of the sac is now labeled primary lymph node, and the base is lettered *b*. The actual size of the triangle as a whole is not greater than when the embryo was 3.6 cm. long. That is to say, the distance from the apex to the base is about 4 mm. in either case.

The ducts over the shoulder from the apex of the sac are well injected, these being the first set to develop. The set of ducts which grows forward from the apex of the sac over the surface of the sterno-cleido-mastoid muscle to make the long plexus in the neck shows somewhat, but is not as well injected in this specimen as in Fig. 7. They are present at this stage but the injection from the region near the eye was not pushed quite far enough to bring them out well. This set of ducts develops into the long and abundant plexus which follows the course of the external jugular vein as it lies parallel to the sterno-cleido-mastoid muscle. From this long plexus, the entire face, front of the neck, fore leg, and thorax are supplied with lymphatics, and these different sets can be seen in Fig. 6. All of these sets of ducts anastomose in the skin, as can be seen in Fig. 5, Vol. III, p. 188.

In brief, the ducts for the shoulder and back of the head grow directly from the lymph heart; those for the face, neck, and fore leg grow from the lymph sac, but form a long plexus along the external jugular vein before reaching the skin. As has just been said, both sets of ducts, distinct in the depth, anastomose in the surface. The line of growth of the lymphatics has been tested by a large number of injections in every stage from the time the lymphatics first appear up to the time of birth. Every injection made into the ducts of the skin of the anterior part of the body will run to the lymph sac or the gland derived from it, if pushed far enough. The different systems of ducts of the neck can be brought out by injecting in four different places. When the needle is entered over the shoulder the injection mass invariably runs to the apex of the

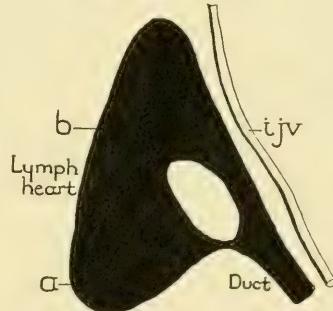


FIG. 4. Diagram of the anterior lymph heart in an embryo pig, 3.6 cm. long. $\times 10$. *a*, apex of the heart; *b*, base of the heart and level of Fig. 5; *i JV*, internal jugular vein.

lymph sac; occasionally it enters the surface ducts that anastomose with the long plexus. When the needle is introduced into the layer of the lymphatics between the eye and ear, or over the lower jaw and front of the neck, or into the pads of the fore feet, the injection mass runs into

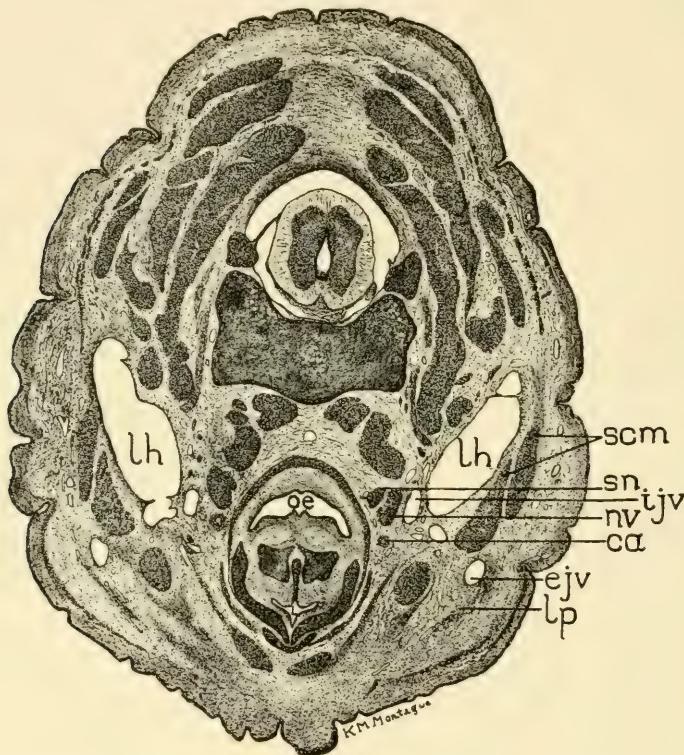


FIG. 5. Transverse section through the neck of an embryo pig, 3.6 cm. long. \times about 11. The shape of the entire heart of which this figure shows a section is given in Fig. 4, in which the line *b* is the level of Fig. 5. *Ca*, carotid artery; *ejv*, external jugular vein; *ijv*, internal jugular vein; *l*, larynx; *lh*, lymph heart; *lp*, lymph plexus along the external jugular vein; *nv*, vagus nerve; *oe*, oesophagus; *scm*, sterno-cleido-mastoid muscle; *sn*, sympathetic nerve.

the long plexus and across the sterno-cleido-mastoid muscle to the apex of the lymph sac. This general relation is not only true in the stages already pictured, but in the later stages when the apex of the lymph heart is a lymph node and the long plexus has been replaced by a chain of lymph nodes.

As is seen in Fig. 6, the ducts which connect the lymph sac and the long plexus, join the plexus half way between the ear and the fore leg. Once or twice, out of many injections in which the needle was introduced between the eye and ear, the injection mass has reached the veins in two ways: one the usual course through the lymph heart, and secondly, through ducts that follow the course of the external jugular vein to its junction with the internal jugular, showing that the ducts along the two veins anastomose.

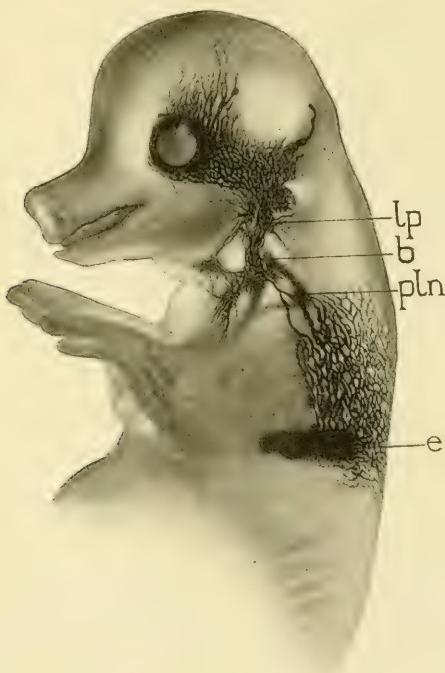


FIG. 6. Lymphatics in the neck of an embryo pig, 6 cm. long, showing the modified lymph heart in the depth and the plexus of lymphatics along the external jugular vein. \times about 3. *B*, lymph node developing in the base of the lymph heart; *e*, extravasation at the point of injection; *lp*, long plexus of lymphatics along the course of the external jugular vein; *pln*, primary lymph node developing in the apex of the lymph heart.

Fig. 7 is from a pig 11 cm. long and shows an injection of the lymphatics made from two points: one between the eye and the ear, and the other into the foot pad. The injection mass, both from the ducts of the face and from the fore leg, has entered the long plexus and then

passed through ducts that lie over the sterno-chleido-mastoid muscle into the node representing the lymph heart (*pln*). At this stage there are two lymph nodes at the angle of the jaw, *nf*, one deeper, receiving the ducts around the eye and cheek, the other more superficial, receiving the ducts just in front of the ear. The rest of the long plexus is also being modified into lymph nodes, one of which is in the middle of the plexus where the ducts join with the lymph sac, the other is at the posterior end of the plexus and drains the fore leg (*nfl*).

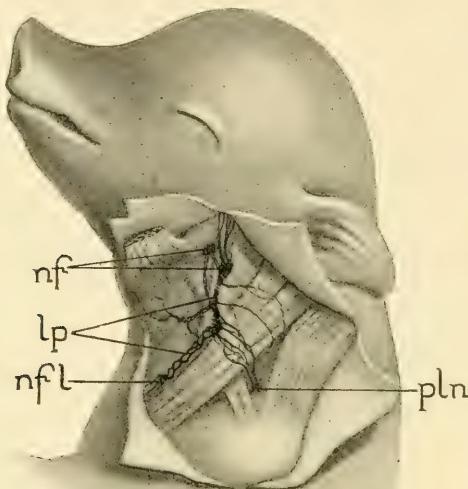


FIG. 7. Lymphatics in the neck of an embryo pig, 11 cm. long. $\times 1\frac{1}{3}$.
Lp, lymph plexus which lies parallel to the external jugular vein; *nf*, nodes developing in the long plexus draining the face; *nfl*, node developing in the long plexus and draining the fore leg; *pln*, primary lymph node between the trapezius and sterno-cleido-mastoid muscles.

Since the spread of the superficial lymphatic capillaries in the skin of the pig is practically complete when the embryo is 6.5 cm. long, it may be well to sum up the superficial lymphatic system at that stage. In the neck there is, in the depth, the lymph heart now considerably modified by the formation of lymph nodes. It has one large efferent duct along the internal jugular vein and one along the main superficial vein of the shoulder. Secondly, there is a plexus of ducts along the external jugular vein; this plexus connects freely with the lymph heart. Lymphatic nodes in the neck are to be found developing first from the lymph sac, secondly in the long plexus on the course of the external jug-

ular vein, and thirdly in the depth along the internal jugular vein. In the surface the capillaries have grown from the apex of the lymph heart to the shoulder and back of the head and from the long plexus to the face, neck, thorax, and fore leg. The capillaries of all these sets of ducts anastomose freely in the skin and there are no valves to check the spreading of an injection mass. The lymphatics of the axilla belong to the deep set which grow along the arteries rather than the veins.

The spreading of the lymphatics for the lower part of the body can be constructed from Fig. 5, Vol. III, p. 188. The position of the posterior lymph heart is just caudal to the kidney, and at this point a lymph node develops. The superficial lymphatics for the lower part of the body grow in two directions, one set following the vein to a point over the crest of the ileum, where a node is formed which drains the skin of the back and hip; the other set coming to the surface in the inguinal region where a long node is formed which drains the abdominal wall and the hind legs. These three nodes with the abundant chain of nodes along the aorta represent the distribution of the glands which drain the skin of the lower part of the body. In following the histogenesis of the lymph nodes all of these different nodes have been studied, but most of the figures presented are from different stages of one node, namely, the first one to develop in the body.

Histogenesis of the primary lymph node.—We turn now to the histogenesis of the lymph node, which will involve determining the structural unit, and tracing the two elements, the vascular element with the adenoid tissue, and the lymphatic element or sinus.

The first lymph node in the body develops from the apex of the lymph heart and will be referred to as the primary lymph node. This node will be traced in its development until its condition is practically adult.

The first evidence of the formation of lymphatic nodes occurs when the embryo is 3 cm. long. At this stage the lymph heart, which has been a smooth walled sac, as shown in Figs. 3 and 5, lined with a single layer of endothelial cells, begins to show a slight modification at the apex in that bands of connective tissue begin to push into the lumen without destroying the lining. The apex of the lymph sac is pictured in Fig. 8 from a pig 3.6 cm. long. The section is taken from the same series as Fig. 5, by which the transverse plane of the section can be noted. The figure shows the character of the surrounding tissue consisting of a syncytium of protoplasm with nuclei in the nodes. The wall of the sac consists of a single layer of endothelial cells, and in the left hand side there is no perceptible modification of the connective tissue. On the right

side, however, bands of connective tissue project into the sac without destroying its endothelial lining at any point. The connective tissue in these bands and on the right border of the sac appears different from the surrounding tissue.

Studied with the oil immersion lens, the surrounding connective tissue appears as described by Mall⁶ to be a network of granular protoplasm in

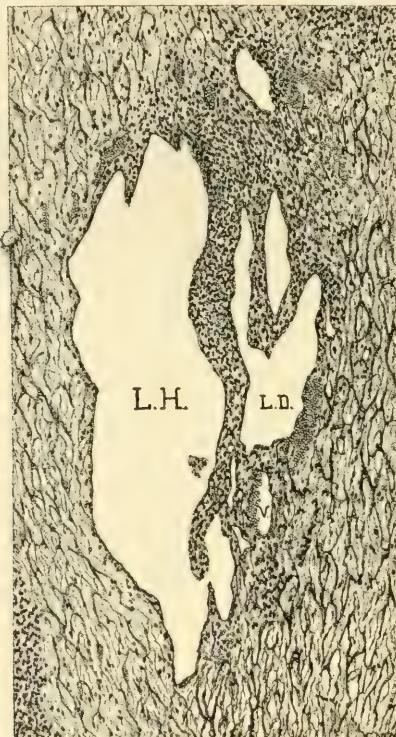


FIG. 8. Primary lymph node from a transverse section of the neck of an embryo pig, 3.6 cm. long. \times about 60. The left side of the figure is the mesial side of the heart, the right side is toward the skin and shows the afferent ducts. The top of the figure is the position of the hilum of the node. *Ld*, lymph duct (afferent); *lh*, lymph heart; *v*, vein.

which are distinct anastomosing fibrils. The nuclei lie in the nodes of the network and each one has around it a drop of clearer protoplasm which he calls endoplasm, distinct from the rest or exoplasm. Near the lymph sac, on the right hand side in the figure, are numerous blood capillaries and around each one are clumps of from 8 to 20 nuclei. These

⁶ Mall: American Journal of Anatomy, Vol. I, 1902.

nuclei lie definitely within the syneytium and belong to the connective tissue. They are only to be distinguished by the fact that they are in clumps and that some show karyokinetic figures while others are smaller and take the deep stain of a newly divided nucleus. In short, cell proliferation takes place around the capillaries.

Passing now to the bands or bridges of connective tissue, the first point to be noted is that there are numerous blood capillaries filled with red blood cells, many of them nucleated. The same sort of protoplasmic network is present as in the surrounding tissue, but the network is denser and the meshes finer. The increase seems to be in the granular protoplasm rather than in the fibrils. In this dense network of protoplasm are crowded many connective tissue nuclei; the mature ones are oval in shape and take the stain faintly. Many of the nuclei are dividing, and there are numerous small, round, deeply staining, young nuclei. These round nuclei belong, however, to the connective tissue and there are no true wandering cells outside of the blood-vessels. Thus the modification of the tissue around the sac consists merely of an increase in the blood capillaries, and in the connective tissue protoplasm and nuclei. The cell increase does not take place independently of the blood capillaries. There is no muscle in the wall of the sac at any time. By the time the embryo is 3.6 cm. long a second node is just beginning at the other end of the sac. This second node from the lymph sac develops in the same manner as the first, but slightly later.

We pass now to the primary lymph node when the embryo is 4.9 cm. long, as shown in Fig. 9. The section is taken in the same plane as Fig. 8, that is, it is from a set of transverse sections. The efferent ducts are on the right and the hilum at the top of the section. There are no striking changes between this and the preceding stage. The node as a whole has increased considerably in size. The lymph heart is about the same actual size as in Fig. 8, but the lymphatic plexus is greater. From Fig. 1 it can be seen that when the ducts first start out from the sac they grow directly to the skin, but in Fig. 9 there has been an anastomosis or plexus formation of the ducts on the border of the sac. This greatly extends the area of the node. On the left side of the sac there are a few blood capillaries with clumps of dividing nuclei around them. The bands of connective tissues show the same abundance of blood capillaries and increase in the protoplasm and nuclei. Young and dividing nuclei are abundant, but no true wandering cells are present.

A more important stage is met with when the embryo is 7 cm. long. From this stage on, the development of the primary lymph node is shown

in a series of five diagrams. Each diagram is made from a single section traced with the aid of the camera lucida. The blood-vessels are put in freehand from the study of the complete set of serial sections from which each diagram was made. All the figures are of the same magnification,

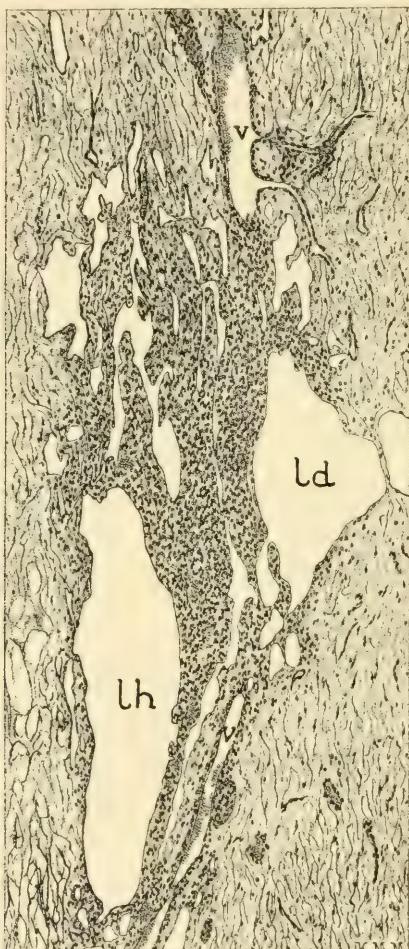


FIG. 9. Primary lymph node from a transverse section of the neck of an embryo pig 4.9 cm. long. \times about 44. The section is placed similar to Fig. 8. *Ld*, afferent lymph duct; *lh*, lymph heart; *v*, vein.

about 33 diameters. The lymphatic vessels are shown in solid black as if injected, while the connective tissue of the lymph cords and follicles is dotted. In the later stages the increased number of the dots represents the increase in lymphocytes and the lines show the beginning capsule and trabeculae.

In the first diagram, Fig. 10, the step in advance beyond the stage of Fig. 9 is in the proliferation of the lymphatic capillaries. The sac has been completely cut up into ducts. The entire node consists of a plexus of lymphatics which differs in no way from the plexus in the skin pictured in my first paper. There are the same swollen bulbs, the same blind sprouts and slender channels. The connective tissue bridges are similar to those of the preceding stages. They contain many dividing cells but no true wandering cells. In the bridges is an abundant plexus of blood capillaries which are not shown in the diagram. This diagram might also represent any lymph node which develops in a plexus.

To sum up, the figure marks the culmination of the first stage of the development of the lymphatic nodes in early embryos, namely, the stage in which the node consists of a plexus of lymphatic capillaries separated by bands of connective tissue which is denser than the surrounding tissue. This stage is shown in Kling's⁷ models, Fig. 1. The connective tissue is embryonic in type, consisting of a net work of granular protoplasm with a few fibrils and with many nuclei. The bands or bridges have blood capillaries and the increase in connective tissue does not take place independently of them. There are no true wandering cells outside of the blood capillaries. It is the stage of lymphatic ducts and pure connective tissue bridges. All of the nodes of the early embryos, the primary nodes in the sense of Gulland pass through this stage. That is to say, the nodes which develop in the long plexus in the neck from which ducts radiate to the face, neck, fore legs and thorax (Fig. 6); or the node which comes in the inguinal region at the point where the ducts radiate over the abdominal wall and hind legs; or in the node over the crest of the ileum where they radiate over the back (see Fig. 5, Vol. III, p. 188). All of these nodes come in places where plexuses are formed because ducts radiate over a wide area, which is shown well in the figure just quoted. They are primary nodes in the sense of Gulland because they develop early and drain large capillary areas. It will be shown subsequently that lymphatic



FIG. 10. Diagram of the primary lymph node in an embryo pig 7 cm. long. \times about 33. The lymphatics are in solid black and the connective tissue bridges are dotted.

⁷ Ibid

nodes which develop later in the life of the embryo, after lymphocytes occur, hurry through the primary process, and show a considerable modification of it.

Up to this time the node has had none of the structures characteristic of the adult node; there are no lymph cords, nor germ centers, no lymphocytes, and no sinuses.

The next stage, pictured in Fig. 11, shows the beginning of some of these structures. The diagram is made from a section of the primary lymph node in a pig 8 cm. long. In the center of the node the blood capillaries have proliferated, giving a tuft of capillaries surrounded by

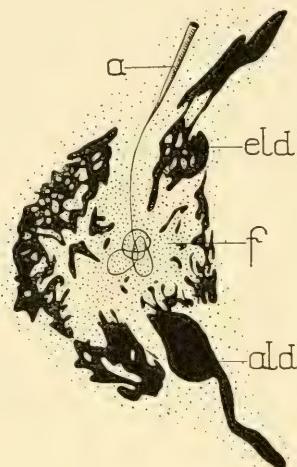


FIG. 11. Diagram of the primary lymph node in an embryo pig, 8 cm. long. \times about 33. This represents the primordial follicle. The hilum is marked by the artery. *A*, artery; *ald*, afferent lymph duct; *eld*, efferent lymph ducts; *f*, follicle.

connective tissue. The artery is shown leading up to the node but reduced to capillaries on entering it. The vein is not shown in the diagram, but the artery and vein lie parallel, up to the point where the node or follicle is entered, where they separate. This is an important and characteristic point in the relation of the blood-vessels. At this stage there are only capillaries within the node.

Here for the first time we can speak of the lymph follicle, which is the vascular unit and consists of the structures that go with a single artery. At this stage the entire node is one follicle. Here also for the first time two elements are differentiated, a lymphoid element connected with the artery and a lymphatic element made of lymph ducts.

The point of entry of the artery determines the hilum of the node. The position of the hilum is determined from the beginning of the formation of the node by the lines of growth. Blood-vessels and lymphatics grow from the center of the body to the periphery, so that the proximal surface of the gland has from the start the entering blood-vessels and the efferent lymphatic ducts, while the peripheral surface of the node is the place from which the efferent lymphatic ducts radiate to the area they are to drain. In the central core of connective tissue the lymphatic capillaries are reduced in number and size; they are never quite absent but do not appear except in well-injected specimens. The presence of these ducts within the connective tissue core may have some bearing on the pathology of lymph nodes. The disappearance of the lymph capillaries in the center of the node involves the retrogression and absorption which is characteristic of developing tissues. Throughout the evolution of the lymph node there is continual building up and tearing down. This will be evident in later stages where there is a continual change in the proportion of the lymphoid structures or cords and the lymphatic structures or sinuses.

Beside being the stage which marks the beginning of the adult structures of the node, that is to say, of the follicle, this stage also shows fundamental changes in cell differentiation. It marks the beginning of the wandering cell in lymph nodes. Up to this time the connective tissue part of the node has consisted of a network of granular protoplasm with many nuclei, young, dividing, and old. At this time three types of wandering cells appear, the lymphocyte, the polymorphonuclear form, and the eosinophile.

Lymphocytes are present in the thymus at a much earlier stage, they are abundant there in the sections from the embryo 3.6 cm. long. In the sections of the lymph node at 8 cm., there are a few lymphocytes in the connective tissue core of the node, and in little clumps in the connective tissue just without the node. These little clumps of cells are found near the capillaries. The differences between the connective tissue cell and the lymphocyte are as follows: The nucleus of the former is large, faintly staining, and oval in shape, and the protoplasm belongs definitely to the network, while the latter has a small, round, deeply staining nucleus, with a more distinct nuclear membrane. The nuclear network and the chromatin granules are coarser, and there are one or more nucleoli. Moreover, the protoplasm makes a narrow but definite rim around the nucleus. Between the connective tissue cell, especially the young forms, and the lymphocyte one can see every possible transition.

Often the connective tissue nuclei appear as if being extended from the protoplasmic network of exoplasm, the irregular endoplasm still clinging to the nucleus.

This form of observation cannot be considered as proof of the origin of the lymphocyte from connective tissue—it is obvious that the position of a wandering cell cannot give evidence of its origin. With the same type of tissue to examine, Gulland, noting the occurrence of the lymphocytes in clumps around the capillaries, concluded that they were filtered from the blood stream. The evidence does not suffice to prove either that the lymphocyte develops from the connective tissue in the lymph node, nor that it reaches the node through the blood stream. We must await some new method of attacking this problem. One point is, however, definite in my specimens—that cell division in the connective tissue takes place in little clumps around the blood capillaries, and, as will be shown later, the division of the lymphocytes takes place also around tufts of capillaries.

Besides the lymphocytes there are a few polymorphonuclear cells at this stage, perhaps not more than twenty or thirty in each section. They are quite typical, having irregular nuclei and finely granular protoplasm. They occur within the follicle. Eosinophiles appear also for the first time. Within the follicle there are numerous red blood cells outside of the capillaries, showing signs of degeneration, that is, a vacuolization and a breaking up of the protoplasm into granules. These granules are all of the same size and cannot be distinguished from the granules of the eosinophilic cell. This is the same evidence that has led Weidenreich to the conclusion that the eosinophilic granule comes from the red blood cell. It is suggestive, but not conclusive.

To sum up the stage represented by Fig. 11, it marks the beginning of the differentiation of the node into its two elements, lymphoid and lymphatic. It shows the beginning of the follicle and of the wandering cell. There is a marked proliferation of the blood capillaries and a consequent increase in the connective tissue in the center of the node. This involves a retrogression or destruction of some of the lymph ducts. At the same time wandering cells appear, lymphocytes in greatest numbers and also polymorphonuclear leucocytes and eosinophiles. There is also evidence of degeneration of the red blood cells.

The next stage is shown in Fig. 12. It was made from the primary lymph node of a pig 13 cm. long. The first point to be noted is the development of the artery. Without the limits of the node, the artery has divided into two branches. These two branches enter the node and

divide into five main branches and two much smaller ones. Consequently there are five definite primordial follicles, and two small ones. Both of the small ones and two of the large ones show in the section. At this stage there is no definite capsule, the limits of the node being determined by the lymphatic vessels. The nodes increase in size by invading the surrounding tissue, for example, the artery which here branches without the node is subsequently included in the gland. This stage marks several important changes. The first has already been noted as being the division of the artery and the corresponding multiplication of the fol-

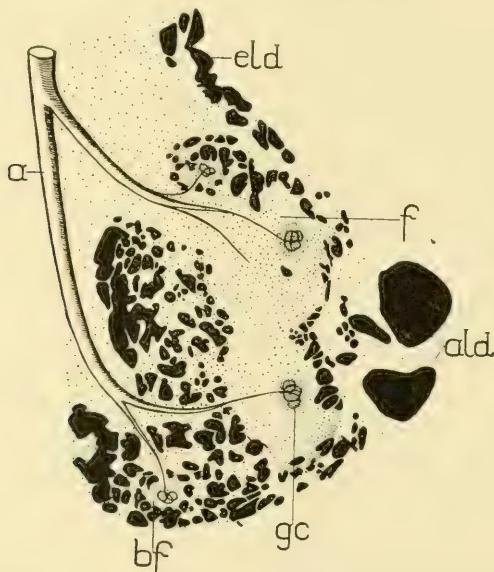


FIG. 12. Diagram of the primary lymph node in an embryo pig, 13 cm. long. \times about 33. The section shows two large follicles and two small ones. *A*, artery; *ald*, afferent lymph ducts; *bf*, beginning follicle; *eld*, efferent lymph duct; *f*, follicle; *gc*, germ center.

icles. The vein, which is not shown in the diagram, runs parallel to each branch of the artery up to the point where the artery enters the follicle. On entering the follicle artery and vein separate and both break up into plexuses. At this stage the artery without the follicle can be distinguished by a thickening of the connective tissue around, there being no media, and by its smaller caliber. The vein has only a lining of endothelium and there is as yet no thickening of the adventitia. Within the follicle, the vessels are all capillaries, but the plexus which connects directly with the artery is made of smaller vessels than the plexus which connects with the vein.

The second point in advance is the formation of the germinal center. Within the follicle, as will be seen in the diagram, there are small clumps of cells, definitely lymphocytes, heaped around a capillary tuft. In the entire node at this stage there are eight of these germinal centers. The lymphocytes are closely packed in them, and there are more lymphocytes near these centers than elsewhere in the node. In regard to the wandering cells, the follicles contain in general four types: First, the lymphocytes which are found in the germinal centers almost to the exclusion of any other free cell. A few of them are to be seen near the germ centers. Second, polymorphonuclear forms, which are scattered throughout the follicle except in the germ centers. Third, eosinophilic cells. Fourth, mononuclear forms which have larger nuclei and more protoplasm than the lymphocytes. All of these cells are found in the follicle. Red blood cells are also present, many of them being free in the connective tissue meshes and showing a breaking up of the protoplasm into granules. This appearance of the red blood cell is seldom seen in the corpuscles within the capillaries. The bridges of connective tissue between the lymph ducts have fewer wandering cells than the follicle.

Beside the two changes already noted, namely, the division of the artery and consequent multiplication of follicles, and secondly, the differentiation of the follicle into germ center and lymph cord, there is a third important change, namely, the beginning of the formation of lymphatic sinuses out of the lymphatic duets. It will be noted in the diagram that around the border of the follicle the lymph ducts are arranged in rows closely packed together and that the connective tissue bridges between them are slender. This is still more definite in the next diagrams given in Figs. 13 and 14. In section, this point is to be seen in Fig. 15, where the surface of the node adjacent to the capsule is made of a plexus of lymph ducts, while in the depth of the node the ducts are closely packed together in certain areas making sinuses. The section shows all gradations in the width of the connective tissue bridges, some being just wide enough to contain a single nucleus while others are broad bands.

Kling was, I think, the first to note this method of the formation of the sinus, though he clouded the clearness of his picture by saying that subsequently the bridging of the sinus is made by the stretching of endothelial cells across the lumen of the ducts, so that there are bridges made of endothelium alone. This appearance is undoubtedly found in sections just as in sections of the lung the epithelial lining of an air sac sometimes shows as a membrane.

By following the evolution of the sinus it is possible to understand clearly the relation of the reticulum of the adult sinus to the endothelial

cells. The reticulum fibers develop subsequently in the connective tissue bridges (not, as Kling says, from the endothelium). The lymph ducts from which the sinus is made have a complete endothelial lining. Moreover, the increase in the ducts which form the sinus takes place by the same process of the budding of endothelial cells which characterizes the development of lymphatics or blood-vessels elsewhere. Thus the spaces of the sinus are lined throughout by endothelium. The sinus can be pictured in three dimensions by imagining the follicle surrounded by a plexus of ducts so dense that the bridges between them are reduced to the thickness of a single network of fibers. Such a structure in cross section would give the appearance of fibers with endothelial cells around them cutting the lumen of the sinus. As a matter of fact the fibers are between the endothelial cells and without the lymph channels. They connect with the rest of the connective tissue framework of the node as is readily seen in Fig. 12.

The next stage is from a section of the primary lymph node of an embryo 15 cm. long (Fig. 13). There is now a great development of the artery. The wall of the artery has developed considerably and shows one row of smooth muscle cells beside the adventitia. The vein which lies beside it has only an endothelium and a thickened connective tissue sheath. The artery divides into three main branches on the edge of the node, and within the node each branch subdivides several times. As in the early stage, the artery and vein run parallel until the follicle is entered and there they separate. The amount of lymphoid tissue has increased parallel with the development of the artery. The sinuses are growing down into the node between the arteries, thereby surrounding and limiting the lymphoid masses around the blood-vessels. By this process the lymph cord along the blood-vessel becomes defined, as will be clear in the next diagram.

The especial advance in this stage lies in the connective tissue, for here the reticulum fibers within the node first begin. Up to this stage the connective tissue of the node has been a protoplasmic network with delicate anastomosing fibrils which, however, do not stain sharply by Mallory's method. Now for the first time there are a few fibrils which stand out clearly in the Mallory stain. They occur in the germ centers where they are laid down in concentric circles. With the oil immersion lens it can be seen that the fibers of the germ centers make a definite mosaic of polygonal spaces in concentric rows. All of these polygonal spaces thus outlined are filled with cells. This appearance of the mosaic can be seen in thin sections of the adult node and can be brought out by silver nitrate.

The diagram shows the first beginning of the trabeculae in the connective tissue that pushes down between the peripheral sinuses as these surround the follicles (see *dt* on the figure). Neither the capsule nor the trabeculae have fibers different from the surrounding connective tissue at this stage.

There are certain interesting points in regard to the cells of the node at this stage. In the germ centers there is a marked division of the

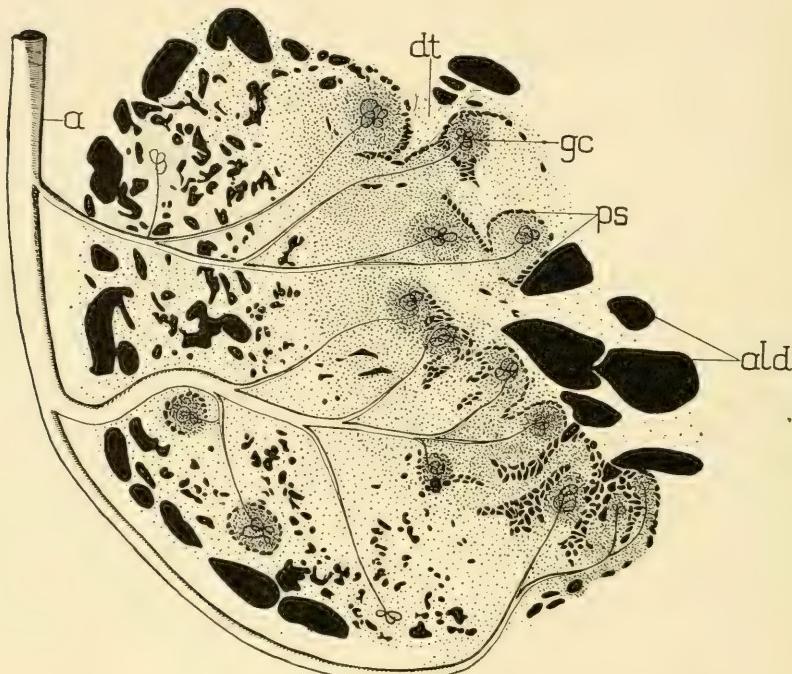


FIG. 13. Diagram of the primary lymph node in an embryo pig, 15 cm. long. \times about 33. The node shows several follicles. *A*, artery; *ald*, afferent lymph ducts; *dt*, developing trabecula; *gc*, germ center; *ps*, peripheral sinus.

lymphocytes. In any one section each center contains from two to fifteen or more dividing lymphocytes. They are easily distinguished from the dividing connective tissue nuclei, which are always larger and have much more protoplasm. There are many eosinophiles in the cords, but at this stage none are to be found in the germ centers. There are numerous degenerating red blood cells.

The next diagram (Fig. 14) is from the primary lymph node in a pig 23 cm. long. Only a portion of the section was drawn, in order to

keep the diagram at the same magnification as the others. An outline of the entire section is shown in the margin. It gives the artery and a few efferent ducts at the hilum and also a large trabecula (*t*), carrying afferent ducts. This trabecula connects with the cortex in another section of the series. The left hand part of the section shows a consid-

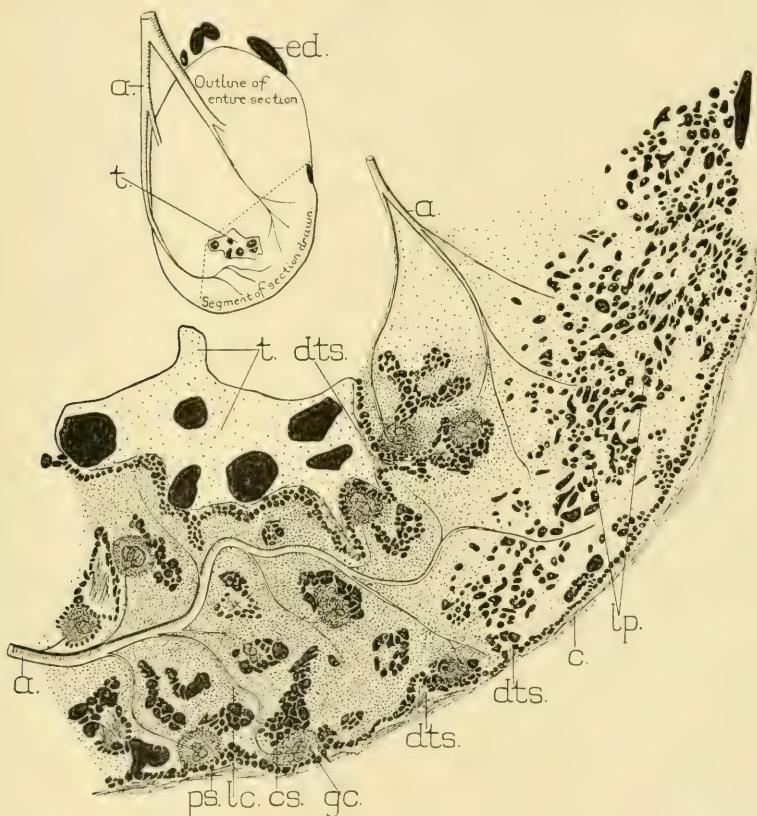


FIG. 14. Diagram of the primary lymph node in an embryo pig, 23 cm. long. \times about 33. The diagram was made from a segment of a section, the outline of the entire section being given in the margin. *A*, artery; *c*, capsule; *cs*, central sinus; *dts*, developing trabeculae of the sinuses; *ed*, efferent duct; *gc*, germ center; *lc*, lymph cord; *lp*, lymph plexus; *ps*, peripheral sinus; *t*, trabecula with afferent ducts.

erable advance over the preceding stage; it represents practically adult conditions where the entire node has been transformed into lymph cords and sinuses. This change has come about in the following manner: The lymph duct plexus or sinus, which was only on the edge of the preceding section, making the peripheral sinus, has now grown into the

depth between the arteries, thereby extending the sinuses and definitely limiting the lymph cords. Indeed, the true lymph cord of the adult condition now appears for the first time. The diagram shows well the nature of the lymph sinuses consisting of rows of closely packed lymph ducts. The bridges between them are still protoplasmic and slightly wider than they appear in the adult nodes.

The diagram shows the relation of the trabeculae to the capsule and the sinuses. Here for the first time there is a definite capsule and it is interesting to note that it is not complete. It extends along the margin of the peripheral sinus but ends abruptly on the right hand side of the section, where the node consists of a plexus of lymph ducts. It will be noticed that there is a definite indentation in the margin of the node where the capsule ceases. At this place the node can increase in size by invading the surrounding tissue.

The structure of the capsule itself is best studied in good Mallory specimens with the oil immersion lens. For this study it is necessary to note the condition of the surrounding connective tissue. This has been described by Professor Mall.² There is in the first place in the surrounding tissue a delicate network of fine but definite anastomosing fibrils. The nuclei of the network are in the nodes, and most of them form part of the characteristic spindle cells. This is the prefibrous tissue of Mall; the fine fiber network is still slightly granular, representing the protoplasmic syncytium of the earlier stages. Most of the definite protoplasm is around the nuclei. Beside this fine fiber network there are large bundles of definite fibers. The fibers of these bundles are several times the width of the fibers of the network, and the bundles themselves are often eight or ten times the diameter of a red blood cell in width. Many of these bundles have several spindle cells clinging to them. The large bundles are found near the border of some gland or muscle, while in the less differentiated areas every transition between the fine network and the coarse bundles can be made out. As Mall has shown, these are the white fiber bundles developing from the prefibrous tissue. The fibers of the bundles are close together and are straight. The capsule of the lymph node is different both from the fine network and from the white fibrous bundles. It consists of a dense network of anastomosing fibers. These fibers are larger and sharper than the fine fiber network, but they are much finer than the fiber bundles. They are wavy and closely packed. In other words, they differ from the fine fiber network by being larger, sharper, and more closely packed, and

² Ibid.

from the fiber bundles by running as separate, wavy fibers, having in general the same direction but forming abundant anastomoses. These are obviously the beginning reticulum fibers.

There is one small, fat organ within the capsule. The trabeculae are formed, as can be seen in the figure, by the folding in of the capsule, thereby bringing the trabeculae in the center of the sinuses (*dts*). This shows especially well in the border of the large central trabecula (*t*) of the figure and explains why the trabeculae of the adult node are bordered by sinuses. The trabeculae often carry blood-vessels from the capsule. The tissue around the blood-vessel at the hilum thickens to form trabeculae; these trabeculae follow the veins farther than the arteries for the arteries soon enter the lymph cords. The trabeculae around the blood-vessels are less developed at this stage than the capsule.

The connective tissue framework within the node itself is a definite protoplasmic network with a few fine fibrils which do not stain sharply in the Mallory stain except possibly in the germ centers. The bridges in the sinuses are all protoplasmic at this stage. In other words, the connective tissue framework of the node is less advanced than the surrounding tissue and the reticulum fiber is a later development.

The diagram shows the relation of the lymph cords and the germ centers. As has been said, the cords are first definitely outlined and restricted to the borders of the artery by the development of the lymph sinuses. Some of the cords are narrow and have the single central artery, but the majority have an abundant plexus of blood-vessels. The artery and vein never run side by side in the lymph cord, and the veins leave the cords to enter the trabeculae that grow in from the hilus. At this stage of development only the large veins are in the trabeculae. The germ centers are around the capillary tufts where the lymphocytes actively divide. The lymphocytes then wander down the borders of the artery, filling up the lymph cords. In the adult node, as is well known, the germ centers may present two different appearances. In the one case, the center is uniformly filled with lymphocytes which are actively dividing; in the other case the lymphocytes are crowded to the edge of the germ center, giving the appearance of a dark rim in sections stained in hematoxylin. In the embryo where cell division is active, the germ centers are always uniformly crowded with lymphocytes.

The right-hand part of the section is far less developed. It consists of a plexus of lymph ducts with connective tissue bridges which contain a network of blood capillaries; it is essentially in the stage of Fig. 7, though the lymphatic plexus is more abundant. Most of the nodes in

the pig at this stage have the center made of definite cords and sinuses and the entire periphery made of a plexus of lymph ducts; and this is the condition of the primary lymph node in a pig 2 weeks old. That is to say, there are three kinds of tissue in the nodes: first, lymph cords and germ centers; second, lymph sinuses; and thirdly, plexuses of lymph ducts not yet transformed into sinuses. It is clear that the lymph plexus is a stage in the development of the lymph sinus, and hence is a less highly developed structure.

These points are clearly shown in Fig. 15, which is taken from the inguinal node in a pig 24.5 cm. long. A portion of the capsule is shown which is not as yet a limiting membrane. The outer part of the node consists of a plexus of lymph ducts with connective tissue bridges. The nuclei in these bridges are large and oval. The inner portion of the node, away from the capsule, consists of lymph cords and sinuses. Within the cords the predominating cell is the lymphocyte, with which the germ centers are closely packed. The sinuses are groups of lymph ducts and transitions between the lymph plexus of the edge of the node and of the developed sinus are to be seen.

In the pig the lymph plexus persists up to adult life. In specimens where the lymph ducts are all collapsed, these areas look like masses of connective tissue, hence Delamere⁹ pictures them and calls them homogeneous areas. Ranvier¹⁰ found the same tissue in the mesenteric nodes of the pig, but since his specimens had the lymph ducts distended he called the areas cavernous or erectile tissue. As has been said, in studying large numbers of lymph nodes in fresh specimens one often finds the lymph ducts or sinuses so distended with fluid that all the spaces are rounded. The presence of this lymph plexus not developed into a sinus in the nodes in the pig is, I believe, an important point not only in the study of development but in the understanding of the structure of allied organs. In all of the haemolymph nodes I have seen, there have been three types of tissue, the lymphoid areas, true sinuses, and zones filled with blood but not definitely sinuses. These zones appear like veins not crowded enough to be sinuses.

We have thus followed the development of the primary lymph node which comes from the lymph sac. The stages are, in brief: first, a preliminary stage in which the entire node consists of a plexus of lymphatic capillaries with connective tissue bridges containing blood

⁹ Poirier, Cunéo, and Delamere: *The Lymphatics*, p. 100, translated from Poirier's *Anatomie*.

¹⁰ Ranvier: *C. R. Acad. Sciences*, 1895, p. 800, or *C. R. Soc. Biol.*, 1895, p. 774.

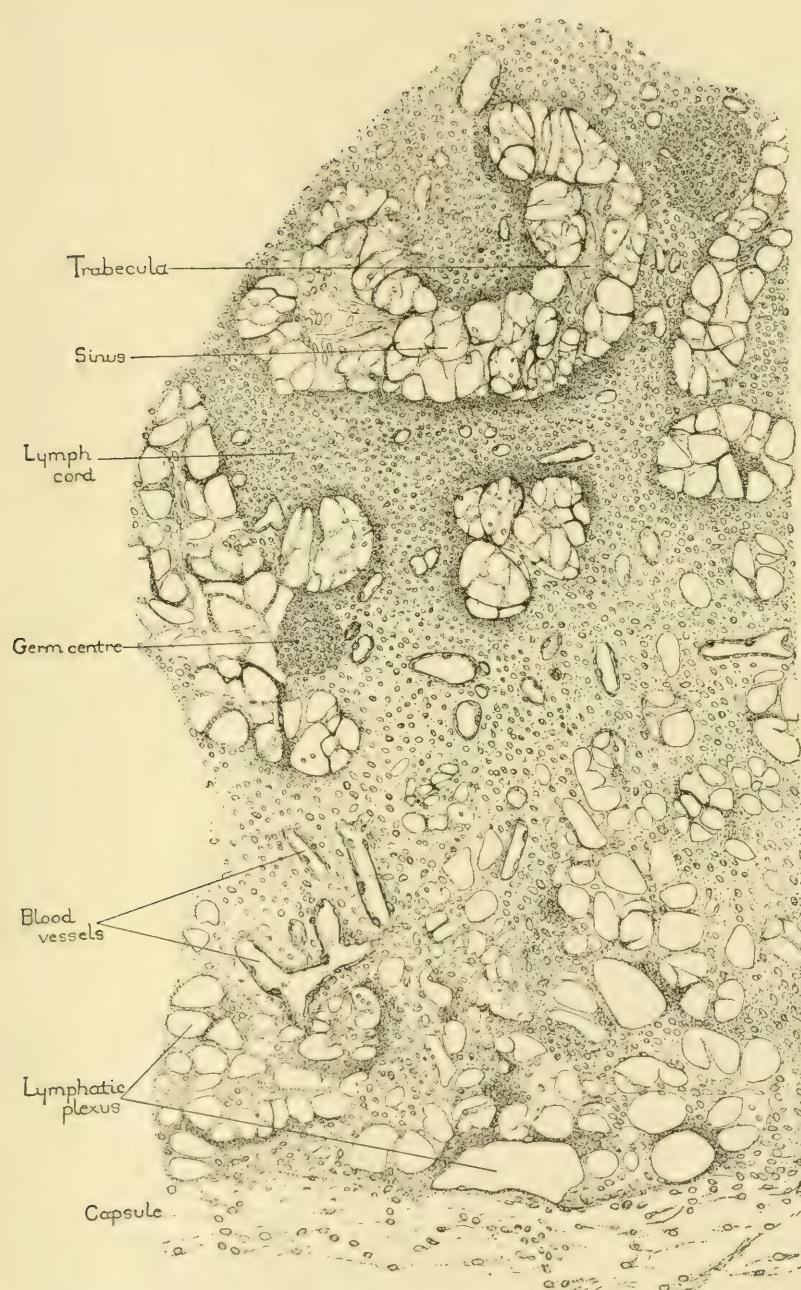


FIG. 15. Portion of the inguinal lymph node in an embryo pig, 24.5 cm. long. \times about 200. Drawn with a camera lucida. The drawing shows the transition between the lymphatic plexus near the capsule and the sinuses farther within the node.

capillaries; then a stage in which the development of the blood-vessels determines the primordial follicle. Following the evolution of the artery the adenoid tissue is determined while the lymphatic part of the node, or the sinuses, develops from the proliferation of the lymph ducts. In the pig the adult node still contains some of the lymph plexus which in higher animals is completely transformed into the sinuses.

Development of nodes in the primary plexuses.—The other early lymph nodes which drain the skin develop in certain definite areas. If we limit the group to those which receive ducts directly from the skin without passing through other nodes, these glands develop in the long plexus around the external jugular vein in the neck and in the plexus of the inguinal region, and over the crest of the ileum. The development of the nodes in all of these areas has been studied. These nodes all begin in a plexus of lymph ducts rather than in a lymph sac; the connective tissue bridges at first show no thickening but soon the nuclei become more crowded and the nodes pass through the various stages shown by the primary lymph node. The node over the crest of the ileum is the simplest, for like the primary lymph node it develops as a single node. The long plexus has the most complicated development and it drains a varied area. In the series at 7 cm. long the entire plexus shows a thickening of the connective tissue bridges. Subsequently, as shown in Fig. 7, the plexus represents a chain of nodes. In this figure four macroscopic nodes are shown, two at the angle of the jaw, one about the center of the plexus, and the fourth at the lower end of the plexus. Of these nodes the one nearest the angle of the jaw receives ducts directly from the skin of the face around the eye; the other node receives ducts both from the skin over the head and from the first node. The node in the middle of the plexus receives ducts from many sources, in fact from all of the other glands in the chain and from the front of the neck as well, while the fourth node receives ducts from the fore leg. Thus it will be seen that some of the nodes are secondary or intermediate in the sense of receiving ducts from other nodes. Some nodes, for example those along the internal carotid artery, receive only ducts that have passed through other nodes, that is, they develop along the efferent ducts of a node, while some receive ducts both directly from the skin and from other nodes. In short, a gland may be secondary for some ducts and primary for others.

The histogenesis of these various nodes follows the same general lines as the primary lymph nodes, beginning with the stage represented in Fig. 10, but there is the widest possible variation in the relative pro-

portions of the lymphoid tissue and the lymphatic tissue. Sometimes the lymphoid tissue, that is, the connective tissue portion with lymphocytes around the artery, nearly fills the node while in other cases the lymphatic ducts predominate.

Development of the follicle independently of the lymph ducts.—One important fundamental point comes out in the study of these

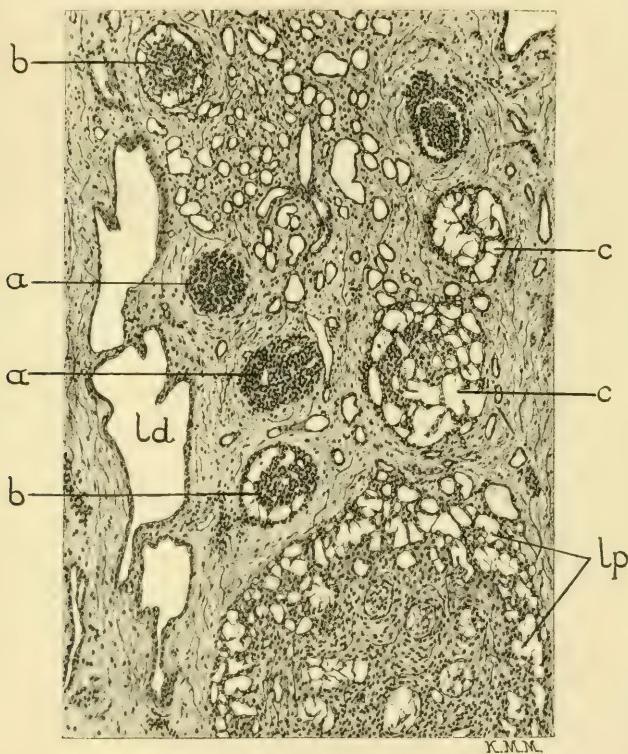


FIG. 16. Group of microscopic lymph follicles in the neighborhood of the inguinal lymph node in an embryo pig, 24.5 cm. long. \times about 72. The condition of the main inguinal node at this stage, is shown in Fig. 14. *A*, follicles consisting of lymphocytes around a tuft of blood capillaries; *b*, follicles with a single peripheral sinus; *c*, follicles which are made mainly of a plexus of lymph ducts; *ld*, large lymph duct; *lp*, lymph plexus in the border of a large lymph node.

various nodes. After a certain stage in development, the large nodes, especially those that develop in the long plexus and in the inguinal region, show many microscopic nodes in their neighborhood. These microscopic nodes are abundant in embryos from 22 cm. long on. Fig. 16 is a section in the border of the inguinal node in a pig 24.5 cm. long,

showing seven microscopic nodes. These different small glands represent all stages in development.

In the figure there is an abundant plexus of lymphatic ducts and around this plexus is a group of small follicles. Two of these nodes, marked *a*, consist of a collection of lymphocytes around a tuft of blood capillaries. Studied through serial sections, these small follicles (*a*) have no lymphatics whatever. Two of the small nodes, marked *b*, have a single peripheral sinus around the follicle while others, marked *c*, have an abundant plexus of lymph ducts not transformed into sinuses. The node in the lower edge of the section is shown only in part. It is the margin of a larger node and shows the peripheral zone of lymph ducts. This entire mass of follicles will be subsequently fused with the large inguinal node, which is one method of increasing the size of the nodes. The inguinal node at birth contains fewer of the smaller follicles in the border than at earlier stages. The study of these microscopic nodes proves an important point, namely, that the lymphoid part of the node or the lymphocytes in the reticulum occurs primarily with the arteries and blood capillaries rather than with the lymphatics. This was suggested by the angiogenesis of the primary node, but is proved by the fact that small nodes are found around the capillaries before they are reached by the lymphatics. That is to say, the follicle is primarily and essentially associated with the artery. That the follicle occurs in the adult without lymphatics is shown in the Malpighian corpuscles of the spleen. In Fig. 17 is a small lymph node found in the lung of an adult pig. It lies in the edge of a lymph capillary but is without a true sinus forming an integral part of the node itself.

To return to Fig. 16, the section shows that nodes which develop at later stages after the lymphocytes occur in the glands, hurry through the long preliminary process of the first nodes; that is to say, they begin at once with a heaping up of lymphocytes around a blood-vessel.

Hæmolymp nodes.—The study of the development of these nodes is incomplete. It has not yet been extended to all the areas in which the hæmolymp nodes occur, but confined to the neck region and along the course of the thoracic aorta. The hæmolymp node does not occur in the neck of the pig until the embryo is about 23 cm long, showing that it is a considerably later development than the lymphatic node. From the time the embryo is 23 cm. long there are one or two small nodes to be found near the node which forms in the center of the long plexus (see Fig. 7). In the specimen at 23 cm. long the hæmolymp node is in the simplest possible stage consisting of a single follicle with a

peripheral sinus filled with blood. It looks exactly like the follicles marked *b* in Fig. 16, except that the sinus is filled with blood. The next stage looks like the nodes marked *c* in the same figure, while a third stage shows an increase in the lymphoid tissue.

The haemolymph node thus parallels the stages in development of the other nodes, except that its sinuses from the beginning belong to the blood-vessels rather than to the lymph vessels. In the lymphatic nodes the sinuses are made of the modified veins called lymphatics, while in the haemolymph node they are made of the veins themselves. The espe-

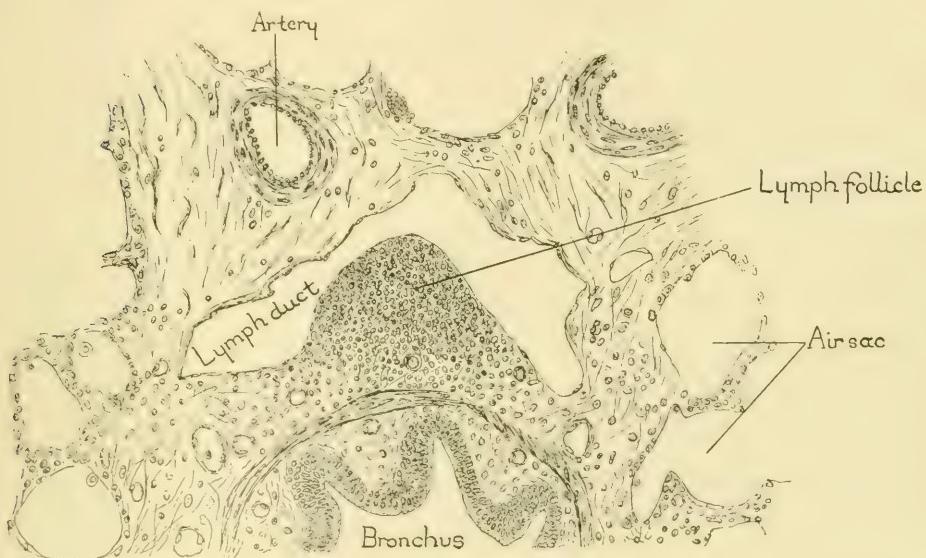


FIG. 17. Lymph follicle without a peripheral sinus, found in the edge of a lymph duct in the lung of an adult pig. $\times 66$.

cial point in the development of the nodes which is not yet clear is the relation of the veins which make the sinuses to the rest of the blood-vessels of the node.

In all of the specimens of the haemolymph nodes as far as they have been studied, that is in pigs up to 3 weeks old, the venous or sinus portion of the node predominates over the lymphoid element. The nodes show both the true sinus and the plexus formation observed in lymphatic nodes, but the developed sinus is much more limited in amount. This is also true in all of the haemolymph nodes I have seen from the adult pig. They consist of three elements, the lymphoid masses surrounded by true sinuses, and a considerable amount of a venous

plexus not transformed into the sinus. Thus the haemolymph node is a later and less developed organ occurring along the blood-vessels.

The haemolymph node found in the neck of the pig is from the beginning a distinct organ, different in type from the lymphatic node. In the adult pig these haemolymph nodes in the neck are sometimes fused into the same capsule with an ordinary lymph node, but the two remain as distinct structures with trabeculae between.

SUMMARY AND GENERAL DISCUSSION.

Follicle.—From the study of the development of lymph nodes we find that there are in general three types of follicles. The simplest follicle consists of a collection of lymphocytes in a reticulum around an artery and its capillaries. This type occurs in the embryo and probably in the adult. The second type consists of the follicle of the first type surrounded by the lymphatic sinus. This type occurs either singly or in groups in the ordinary lymphatic gland. In the third type the lymphoid follicle is surrounded by a blood sinus or a less developed plexus of blood-vessels. This occurs in the haemolymph node and spleen.

Lymphocytes.—In connection with the nature of the lymphoid tissue it has already been brought out that the lymphocyte occurs in a fine reticulum around the artery and its capillaries. For the ultimate origin of the lymphocyte we have as yet no proof, but the lymphocytes divide, as Flemming showed, in the germ centers. The germ centers are definite organs around capillary tufts or glomeruli. In the embryo the division of lymphocytes is so constant that the germ centers are always filled with them. The lymphocyte develops independently from the lymph ducts.

General structure and growth.—In regard to the general structure of the node, it has been shown to consist of two elements—a lymphoid or vascular and a sinus element, which is either venous or lymphatic. Through the study of the development of the nodes, it becomes clear that the sinus comes from a plexus of ducts or vessels by increasing the number of ducts and reducing the size of the connective tissue bridges. In the pig the plexus element of the node is not completely transformed into the sinus in the adult, so that the node has three structures, the lymphoid element, the plexus, and the sinus. There are wide variations in the proportions of these elements.

The question of growth and increase in size of the lymph node is an interesting one, closely bound up with the process of absorption in development. All of the diagrams show that the nodes in the early stages increase in size by the invasion of surrounding tissue by lymph ducts.

This point is brought out by comparing Figs. 5 and 6, from the primary node at 3.6 and 4.9 cm. long. The sections are cut in the same plane and can be related by the sac. It will be seen that the increase in size of the second is due to the development of the lymph plexus. This invasion of ducts can take place as long as there is no definite capsule. The capsule is a late development, as shown in the last diagram, where it is still incomplete. After the capsule is formed the nodes increase in size by the fusion of many small nodes in the neighborhood. The capsules of the smaller nodes become the trabeculae of the larger ones. The marked tendency of the fusion of nodes in the pig has been noted by other observers. In the pig not only do the ordinary lymph nodes fuse, but a haemolymph node may fuse with a pure lymph node and the two remain distinct but enclosed in a common capsule. The primary lymph node from the lymph sac, and the node over the crest of the ileum show but few of the small nodes in the neighborhood and hence few evidences of fusion. On the other hand, the inguinal node which represents a group of nodes in higher forms, appears like a conglomerate of small nodes in the new-born pig.

The lymph nodes give much evidence of a repeated tearing down and rebuilding in the process of growth. For example, in passing from the stage of the first to the second diagram, there must be a destruction of many lymph ducts in the formation of the primordial follicle. It will be noted that this destruction is not complete in Fig. 8, for there are a few lymph ducts scattered through the follicle. In following through the diagrams it will be noted that there is a constant change in the proportions of the lymphoid or vascular portion and the lymphatic portion of the node. The small nodes of Fig. 16 make this point clearer. The youngest ones have a predominance of the lymphoid portion, the very youngest ones having no lymph ducts at all. Others have merely a peripheral sinus, while others slightly larger are made almost entirely of a plexus of lymph ducts. As these nodes develop farther the lymphoid tissue will increase until the balance of lymphoid tissue, sinus, and lymph plexus characteristic of the adult node is reached. This point illustrates the extreme variation met with in the development of the lymphatic apparatus.

Sinus.—The lymph sinus develops out of the lymph ducts by a multiplication of the ducts along certain lines. The areas in which the lymph ducts multiply until the connective tissue bridges are reduced to the thickness of fibers, are determined by the blood-vessels. That is, the sinuses grow in between the arteries thereby bounding the lymph cords.

The sinus develops by a proliferation of the endothelium of the ducts, and so, as Kling pointed out, each space in the sinus has its complete ring of endothelium. The fact that the sinus is made of a great number of small lymph ducts packed closely together explains why one cannot get the silver picture of a membrane of endothelium far beyond the periphery of the node. None of the membranes are large enough. In the embryonic stages the connective tissue bridges are largely protoplasmic and show connective tissue nuclei. In the adult the bridges are a network of reticulum fibrils so that the endothelial cells appear on the fibers.

The study of the contents of the sinuses will prove, I think, an important point in the physiology of the lymph nodes. There is a marked difference between the embryonic nodes and the adult in this respect. In the embryonic nodes there are few free, wandering cells in the sinuses as compared with the adult. In the early stages there are almost no free cells in the ducts. After the lymphocytes appear, they occur occasionally in the ducts and sinuses, as well as a few large mono-nuclear forms. As a rule the sinuses are nearly empty of the free cells, so that the bridges stand out with great beauty and clearness. In the adult node, on the other hand, the content of the sinus in free cells is most varied, both in the number and in the kind of cells. Sometimes the sinuses are so packed with cells that the reticulum and endothelium are almost covered up. It often happens that the sinuses of one portion of a section are densely packed with cells, while in another portion they are almost empty. These free cells may be any type of white blood cell or may be large phagocytic cells. The sinuses may be filled with phagocytic cells that are crowded either with red blood corpuscles or, in the mesenteric nodes, with fat globules.

Reticulum.—The complete relation of the connective tissue framework or reticulum can only be clear after noting the nature of the sinus. The reticulum fibers first appear in the germ centers where they make a mosaic pattern around the capillary tuft. The trabeculae develop from the capsule in connection first with the sinuses and secondly with the blood-vessels, especially the veins. The reticulum fibers are laid down in a very close protoplasmic syncytium, and this syncytium remains protoplasmic long after the surrounding connective tissue has become predominately fibrillar. The reticulum fibers do not show until the embryo is 15 or 16 cm. long. They appear first in the germ centers and in the capsule. In an embryo 23 cm. long they are limited to the capsule and a few trabeculae, while up to the time of birth the connective tissue framework is still largely protoplasmic.

The reticulum framework in the adult makes a complete anastomosis throughout the node. The framework can be readily traced in the last diagram. Starting from the capsule, fibers enter the trabeculae, pass between the ducts of the sinus and enter the lymph cord. The large trabeculae carry veins. In a specimen of reticulum from which the cells have been digested out, the entire node can be reconstructed for the sinuses occur in the looser reticulum that borders the trabeculae while the cords and follicles are between trabeculae and show a much finer and denser network than the sinuses. The germ centers often appear as holes, since the fibers are delicate there.

Lymph capillaries.—The subject of open or closed lymphatics, within the lymph node as elsewhere, has given rise to most definite but opposite opinions. The study of development touches but one aspect of the question, unless it is combined with many injection experiments. The lymphatics develop as blind sacs from the veins and have a complete endothelial lining. The sinuses result from a multiplication of the lymph ducts and the only difference between the sinus and the preliminary lymph plexus is in the width of the connective tissue bridges between the ducts. In the sinus these bridges are reduced to the thickness of the reticulum fibers of the adult. Thus from the purely embryological argument the spaces of the sinuses have a complete endothelial lining. Numerous injections of lymph nodes in every stage have been made with Prussian blue and with India ink. The ink always runs farther than the Prussian blue. In injecting the nodes through the afferent ducts it is always easy to avoid undue pressure within the node, for the lymphatic area of the node is so much greater than the caliber of the ducts leading to it. It is possible in all stages up to two weeks after birth to obtain injections without extravasations. But many injections do show extravasations and these occur in the walls of the sinus rather than the less developed plexus of ducts which always forms a part of the node. This shows that the wall of the sinus is weaker than the walls of the plexus.

To conclude, the present study shows the close relation between the lymphatic system and the vascular system. In the formation of lymph nodes, haemolymph nodes, and spleen, there is one fixed element in common, namely, the lymphoid tissue associated with the artery; the fluctuating element or the sinus belongs either to the venous system or to modified veins called lymphatics. The sinus may be absent, or venous or lymphatic.

ON THE ANGLE OF THE ELBOW.

BY

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WITH 1 FIGURE AND 8 TABLES.

Artists consider a woman's arm beautiful when, in its extended position it is straight, or nearly so, and sufficiently plump to give it delicate curved lines. With the elbow flexed the upper arm is considered the more beautiful the nearer its section approaches a circle. The same is claimed for the forearm near the elbow. As the section approaches the wrist the circle becomes an ellipse, and the farther it is from the elbow the more marked is its eccentricity. With the forearm flexed and semi-prone the long axis of the ellipse is directed diagonally downward and outward. It is self-evident that artists do not make either cylindrical or conical arms; they prefer as a model an arm whose section is nearly circular. The forearm is always a little flat, more so in supination than in pronation. In antique statues the upper arm is found to be more nearly circular, while in those of the renaissance a lateral flattening is shown. It appears then that the ideal arm of artists changes from time to time, possibly because the models before them changed correspondingly. At any rate the shape of the upper arm of the renaissance approaches the modern anatomical arm more than does that of antiquity.

The form of the ideal woman's arm is caused in great part by the layer of subcutaneous fat drawn over the structure below. In the ideal man's arm the structures below, especially the muscles, protrude and form marked lines indicating strength. And it is considered beautiful by many to show some of these lines in a delicate way in woman's arm. A slight outline of the deltoid, biceps and triceps does not make the arm appear masculine provided it is built upon a delicate skeleton.

The beautiful arm, then, is one that is plump, round, tapering and relatively straight. Differences are, of course, to be expected, due to race, sex and age. The amount of fat upon the arm differs much between the ages of 15, 25 and 45. The same seems to be true regarding the angle of the elbow. The arms of young girls are said to be straighter and

the motion of the elbow seems to be greater than are those of young women. At any rate, from an artistic standpoint, a slight amount of hyperextension is permitted in a child's arm, but it makes a bad impression when it is present in the arm of a muscular man. It is evident that the standard of the beautiful must change for different periods of life, and the question is whether the beautiful and anatomical normal correspond. It was mentioned above that the arms of antique statues were unlike in form those of the renaissance, the latter being more realistic.

The Greeks constructed the canon of the human body with its length eight times the length of the head, which was taken as the modulus. This gave a rather short body, too much so, for Michael Angelo found it necessary to add one-third of a modulus, making the body $8\frac{1}{3}$ heads long. This third of a modulus was added to the legs above, which were also extended one-sixth of a modulus below. Thus Michael Angelo's canon is half a head longer than the Greek canon, all of the difference being added to the legs. This may account for the plump arms of the Greeks and the thinner arms of the renaissance. Since that time many systems of measurement have been invented, differing mostly in the modulus, and contributing little to the proportion of the body. It appears that the first scientific step was taken by Quetelet, who drew averages from the measurements of some 30 soldiers. So much variation was encountered, however, that his results proved to be of little value. Others made many measurements, as is shown by Sargent's admirable work on the average figure of American students of both sexes. The next step in advance was made by measuring from the principal joints to obtain the proportions of the trunk, and the most satisfactory system is that of Fritsch, whose modulus is the length of the spinal column. His canon, to a certain extent, outlines the human body, giving at a glance many ratios. When this is compared with numerous recent outlines it is remarkable how well they coincide. It may be added that the best recent outlines of the body were constructed by anatomists and that there is now a tendency for artists to accept a canon which is anatomically correct. Furthermore, this canon is much more like that of antiquity than like that of the renaissance, being half a head shorter than the Greek canon.

The difficulty is not to be solved by inventing a new modulus but by establishing its length. This in turn will establish the length of all other important measurements, bringing ultimately the artistic ideal and the anatomical normal together. Furthermore, measurements from the centers of the main joints are most desirable, for then exact measure-

ments can be made in a statue, since a femur or a humerus measures the same in all positions. Fritsch's canon fulfills this requirement. That the average measurement is the most beautiful is further proved by observing composite photographs of many average faces, few of which are considered beautiful, while together they are decidedly so. Nature has here been and must continue to remain our best standard.

To what extent artists may idealize variations is not for me to consider now. They must, however, remain within bounds, and when they emphasize a variation of one part their convention must make the rest of the body the anatomical normal in order to bring out well the difference. So if straight arms are the artistic ideal, the rest of the canon must correspond with the anatomical normal.

In a model an arm is not considered beautiful if it is too long, for this is said to be indicative of a lower race. Neither is hyperextension nor a lateral angle considered desirable. It is especially inartistic to have the two combined. Hyperextension of the elbow may be overlooked in children and in delicate girls, for it helps to indicate the flexibility of the body, which is a characteristic of youth.

It appears that those who write upon the angle of the elbow from the standpoint of the artist are not altogether familiar with its anatomy, for the straight arm is considered the more usual. According to Brücke, the lateral deflection of the forearm is more common in women than in men, while according to Stratz the opposite is the case.

The latter author makes the normal arm so straight that the wrist in turning rotates in a circle with the radius as its center, thus in pronation the lower end of the ulna moves lateral as much, or more, than the radius does medial (see Stratz's Fig. 67).

It has been known for a long time that the trochlea is not set at right angles to the shaft of the humerus but obliquely to it, making an acute angle directed outwards. It is this lateral angle which, according to Langer, causes the forearm to bend out when the arm is extended, and assuming that the articular surfaces of the ulna and radius are at right angles to the forearm the hand must fall upon the chest when the elbow is flexed. The amount of lateral deflection when the arm is extended equals the extent the hand moves in when the arm is flexed. The observations of Langer are accepted by Brücke, but apparently he does not consider the normal arm beautiful, and suggests various methods by which it can be corrected in a picture or a statue by always presenting the arms either partly pronated, or partly flexed, or both.

The subject was carefully reworked by Braune and Kyrklund in their study of the elbow joint, and they show that not only is the axis of

the elbow joint set obliquely to the humerus but also to that of the forearm. As a rule the angles formed by the axis with the humerus and with the forearm are nearly equal, each measuring about 83° , both acute angles pointing outward. The styloid process of the ulna which marks the long axis of the forearm in both pronation and supination, deflects fully 14 degrees from the sagittal axis of humerus when the elbow is extended, and gradually approaches it as the elbow is flexed, for the angles of the humerus and of the forearm neutralize each other in the flexed position.

This study was made to test these results, and to determine the extent of the motion of the elbow in the European and in the negro, male and female, for during a number of years past I have felt conscious that a sexual difference exists.

In order to make satisfactory measurements fixed points had to be established and after extending Braune and Kyrklund's reliable method a modified method for measurement was hit upon which when applied repeatedly to the same arm gave an error of less than one degree. Unfortunately there are more variations than I had anticipated, but I venture to give my data with the hope that they may be of more general use, and that I may be able to add to them in the course of some years. To collect and measure 100 specimens is not altogether a small task, but this kind of work must be multiplied many fold before the foundations of anatomy—descriptive, regional and artistic—become anthropological.

The arms studied were taken from the dissecting rooms and carefully cleaned, leaving all of the ligaments of the elbow intact. Then the axis of the elbow was determined by fixing the ulna and radius, and moving the humerus to and fro. By doing this it is quite easy to find a point over each epicondyle which does not move. A line extending through these two points passes through the middle of the trochlea and marks the axis of the elbow joint. Frequently there is not an immovable point over one of the epicondyles, but instead a line is determined and in most cases the middle of the line is taken as the axis. Next the humerus was fixed with this axis and a point in the middle of the upper third of the shaft in a horizontal plane 15 cm. above the plane of the table. The perpendicular plane was then passed through the middle of the upper third of the humerus and through the coronoid process, for it has been shown by Braune and Kyrklund that this process keeps within a millimeter of this plane, passing first to one side of it and then to the other. The screw motion which is said to be present by Meissner, Henke and Langer does not exist. Five centimeters from this plane a glass plate was fixed from which the measurements were taken. The horizontal

plane becomes a coronal plane when the arm hangs down along the side of the body, and therefore I still call it the coronal plane. In the same position the perpendicular plane is parallel with the sagittal plane of the body and may therefore be called the sagittal plane of the arm. The intersection of the two planes forms the axis of the humerus passing through the center of the upper third of the shaft and through the axis of the elbow below the coronoid process.

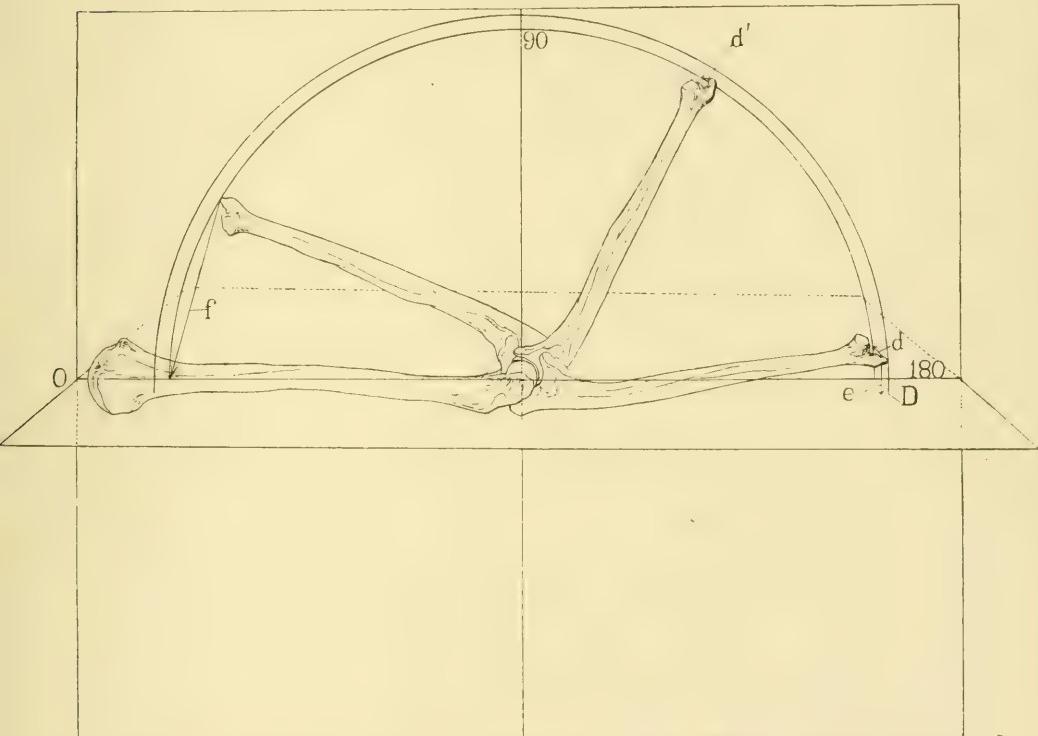


Diagram of the bones of the arm with the planes from which measurements were taken indicated. D , circle of maximum deflection of the ulna; d , chord of the arc of deflection when the elbow is extended; d' , the same at about 110 degrees; e , chord of the arc of extension when reduced to degrees to be subtracted from 180 degrees; f , chord by which the extent of the flexion of the elbow is measured.

The angle of the axis of the joint with the long axis of the humerus was first determined by direct measurement, the right arm being clamped with the humerus to my left, and the left arm in the opposite position. In all cases the degree of flexion, extension and deflection was determined by measuring from the styloid process of the ulna. The degree

of flexion was determined by the chord of the arc which would be described by moving the styloid process from maximum flexion to the long axis of the humerus with the axis of the elbow as the center. The degree of extension was determined by the chord of the arc described by the styloid process between maximum extension and the projected axis of the humerus. Accordingly when the elbow joint did not extend to a straight line or when it hyperextended this amount was respectively subtracted from or added to 180 degrees. The amount of deflection was determined for three positions measuring from the styloid process with the elbow flexed, extended and at 90 degrees. The degree is determined by the chord of the arc described by the styloid process intersecting the sagittal plane at right angles in these three positions named above. In case the deflection is out it is marked plus and in case it is in it is marked minus.

In the appended tables the first column gives the number of the cadaver. The second column gives the length of the ulna from the styloid process to the axis of the elbow joint. Next the angles of the humerus and the ulna with the axis of the arm are given. These, together with the degree of deflection of the forearm with the elbow joint extended to its maximum, always equal 180 degrees. Then follow the columns with the degree of motion, from maximum flexion to maximum extension, 180 degrees being a straight line. The lateral angle is next given in three positions and when it is marked minus it indicates that the arm turns in. This takes place frequently when the elbow is flexed to its maximum, occasionally when at right angles, and not at all when it is extended. In other words, when the arm is extended and supinated the whole wrist including the styloid process lies to the outward of a line drawn through the middle of the upper third of the shaft of the humerus and the coronoid process of the ulna. All arms are deflected laterally.

Braune and Kyrlund have shown conclusively that the elbow joint is a pretty perfect hinge joint and that there is practically no screw motion in it. As it flexes the ulna shifts a little, first outward then inward, which motion causes the shaft of the humerus to rotate outward nearly 6 degrees in case the forearm is flexed. For all practical purposes the joint is a hinge joint, the axis being set obliquely at nearly 84 degrees to both humerus and ulna. In all cases the styloid process of the ulna deflects about 12 degrees when the arm is extended and when flexed because the angles of the humerus and ulna are about equal, the ulna lies in the sagittal plane of the humerus, *i. e.*, the ulna comes to lie directly upon the humerus and not upon the chest as is claimed by Langer. In case the angle of the axis of the ulna is less than that of

the humerus, the styloid process still deflects when the elbow is flexed and in case it is greater it is turned in. Braune and Kyrklund's few cases (nine in number) seem to bear out these statements, but they are by no means always borne out by my records. The sigmoid cavity does not hug the trochlea closely and the slight rotation of the coracoid and olecranon processes may be sufficient to account for my figures. Furthermore, the inequalities in diameter and form of the two conical surfaces of the trochlea may cause sufficient shifting to counteract a slight difference between the angles of the humerus and ulna. This is already indicated when the points below the epichondyles through which the axis passes are determined. One of them is usually extended into a line several millimeters long showing that the section of the cone of the trochlea is not circular on that side. Even my averages do not confirm Braune and Kyrklund's notion. In my 89 specimens, the average of the angle of the humerus is 82.5 degrees, and of the ulna 86.5 degrees, and yet the styloid process still deflects .5 degree when the arm is flexed to the maximum. This, of course, is when the elbow is flexed to 39 degrees, and could it be flexed to 0 the styloid process should turn in about 2 degrees. In general the irregularities of the surfaces of the elbow joint fully neutralize the fine difference between the angles of the humerus and ulna and only in a general way is the assertion of Braune and Kyrklund correct. In about three-fourths of my measurements the angle of the ulna is greater than that of the humerus, while in Braune and Kyrklund's measurements (but one-eighth as many) they were just the opposite.

The extent of motion of the elbow from flexion to extension gives some interesting results. It is well known that the extent of movement in the joint of children is much greater than that of adults, and artists often try to express this in the arms of children and young, delicate girls. I have often observed this difference in examining arms of infants in the dissecting rooms. In fact, in numerous specimens which I have examined not a single infant's arm was found in which the elbow could not be hyperextended. The measurements from the adult arm which I have made give equally interesting results, for they point towards a sexual difference. The following table gives the degree of extension of

Degrees of extension.....	155°	160°	165°	170°	175°	180°	185°	190°	195°
Number of males.....	1	6	19	14	14	6	3	1	..
Number of females.....	2	6	7	5	2	1	2
Total.....	1	6	21	20	21	11	5	2	2

89 measurements. The straight arm is 180°. It is evident that the female arm is straighter and more frequently hyperextended than the

male. The degree of flexion gives a similar table, which becomes more

Degree of flexion.....	20°	25°	30°	35°	40°	45°	50°	55°
Number of males.....	3	17	23	17	3	..
Number of females.....	1	1	6	8	6	3	..	1
Total.....	—	—	—	—	—	—	—	—
	1	1	9	25	29	20	3	1

marked when it is expressed in differences, that is the degree of motion, from maximum flexion to maximum extension.

Degree of motion...	110°	115°	120°	125°	130°	135°	140°	145°	150°	155°
Number of males...	2	5	9	6	15	11	12	2	2	..
Number of females...	1	2	4	8	5	2	3
Total.....	—	—	—	—	—	—	—	—	—	—
	2	5	9	7	17	15	20	7	4	3

The two lines now move away from each other more than before, the greatest number of cases for each sex being 10° instead of 5° apart. In constructing this table the degree given is each time the middle figure; for instance, 130° includes 128° to 132°. Furthermore, there seems to be a slight racial difference which tends to make the sexual difference rather less marked than it really is. The greatest number of European males occurred in maximum extension under 170°, in maximum flexion under 35°, and in degree of motion under 135°. In other words, the motion of the elbow of the European male is more nearly like the female than the male negro. So when the joint of the negro alone is considered the sexual difference is more pronounced than when it is considered with that of the European. The following table

Degree of motion.....	110°	115°	120°	125°	130°	135°	140°	145°	150°	155°
Negro { Number of males....	1	5	7	6	12	7	8	2	1	..
{ Number of females....	1	4	8	4	1	3
Total.....	—	—	—	—	—	—	—	—	—	—
	1	5	7	6	13	11	16	6	2	3

includes only the arms taken from negroes. On account of an insufficient number of records further tabulations give no results which are definite.

As far as the records go, they indicate that the elbow joint of the female is more flexible than that of the male—is more of the infantile type—and that of the European male holds an intermediate position between the negro male and negro female. Practically all of the subjects considered came from the laboring class, so a difference on account of muscular development cannot be entertained.

The amount of deflection of the forearm is shown in the data which follow. In all cases the styloid process deflects when the arm is extended

and every specimen verifies the statement of Langer and Brücke, that the whole wrist falls to the outside of the sagittal plane of the humerus when the forearm is extended and pronated. The assertion of Stratz that in this position the line falls in the middle of the wrist is absolutely incorrect. Furthermore, his diagram (Fig. 67) which is apparently based upon Merkel's normal figure, is also incorrect, for Stratz's own copies of Merkel's figures (Figs. 31 and 32), as well as the originals, coincide with Brücke's as regards this point.

With the elbow extended the average deflection of the styloid process of the ulna from the sagittal plane of the humerus is 11° from my measurements. The average length of the ulna from the axis of the elbow to the styloid process is 258 mm. With these two measurements 11° equals a chord about 5 centimeters long, so the styloid process deflects normally 5 cm. or about the width of the wrist. Therefore, with the arm extended the wrist should fall outside of the sagittal plane of the humerus in both supination and pronation. In both positions the styloid process falls about 5 cm. to the outside of the sagittal plane of the humerus and in pronation it passes through the *styloid process* of the *radius*. In the extended arm all of the wrist, or at least its greater part, falls lateral to the sagittal plane of the humerus in both pronation and supination. This marked deflection, more so in my records than is stated by any author, is no doubt due in part at least to a racial difference, for 70 of the arms are from negroes and but 19 from Europeans. A glance over the tables shows that some difference does exist, which I shall now consider. When the differences in deflection are grouped for every 5° , as was done when discussing the motion of the elbow, nothing definite is noted, and when they are grouped under single degrees the figures scatter so much that it is again difficult to see any marked result. The negro male, however, shows some 3° greater lateral deflection in the movement from flexion to extension than does the European male. The difference between the European male and female is much greater, but the number of cases are so few that this also cannot be considered. It would indeed be remarkable if more records showed that the European female had the greatest lateral deflection and that the European male the least, that of the negro lying between. If it should prove to be so, then artists have secured their ideal straight arm of females from the males and infants¹ where the lateral deflection is the least.

The racial difference becomes more marked when the total amount of

¹ Braune and Kyrklund state that the angle of the humerus in infants is much less than in adults.

deflection (that is, the difference between that at flexion and that at extension) is divided by the number of degrees of motion of the elbow. The deflection being greatest in the negro male, the result becomes still greater because the motion of his elbow is the smallest. This quotient becomes the degree of lateral deflection for one degree of elbow motion. If this in turn is multiplied by 180, the amount of deflection is obtained, in case the elbow joint could be moved from zero to 180 degrees. This quotient I shall speak of as the total deflection, it being the amount of deflection in case the elbow joint had a motion of 180 degrees. For example, in the right arm of subject No. 925 the deflection is between — 4.5 degrees and 1 degree or 5.5 degrees. This divided by the number of degrees of motion (169 — 50) 119 makes .0462 or the number of degrees of lateral deflection of the forearm for each degree of flexion or extension. In turn this multiplied by 180 gives the total deflection could the forearm move through an entire semicircle. In this case it amounts to (.0462 \times 180) 8.3 degrees. Now it is found that the deflection per degree varies for different positions of the forearm, as is shown in the following table. In the first column the deflection per degree is

Race	Sex	Arm	Degree of deflection for each degree of motion from		
			Maximum Flexion to 90°	90° to Maximum Extension	Maximum Flexion to Maximum Extension
Negro	Male	Right08	.10	.09
		Left07	.08	.075
		Both075	.10	.08
	Female	Right07	.09	.08
		Left075	.08	.08
		Both07	.085	.08
		Both075	.09	.085
European	Male	Right04	.07	.06
		Left025	.055	.046
		Both03	.06	.05
	Female	Right11	.10	.10
		Left10	.05	.07
		Both105	.075	.085
		Both05	.075	.065
Average07	.085	.08

given between maximum flexion and 90 degrees, in the second from 90 degrees to maximum extension, and in the third the average degree of deflection for the whole motion of the forearm. Of course to determine each figure it was necessary to start with an average. It is seen from the table that the lateral deflection per degree of motion is generally less when the elbow is flexed less than a right angle than when it is extended beyond it. The deflections seems to increase as the maximum extension is approached. This is to be accounted for in part by the irregularity of

the surface of the elbow joint. When the averages are considered it is seen that there is a marked difference between the deflection in the negro and in the European which becomes more pronounced when the males only are considered. The race of fully 70 per cent of the cadavers from which these arms were obtained can be determined by these measurements. The average deflection here is $.08^\circ$ and $.05^\circ$ for each degree of motion, and this difference is pretty constant, as can be seen from the table. The deflection between maximum flexion and maximum extension for negro males and European males is 10.5° and 6.50° , which, considering the differences in the lengths of the ulnas equals 5 cm. and 3 cm. respectively. If the total deflection is considered, that is, if the motion of the elbow were 180° , the deflection would be 14.5° and 9° for the negro and European respectively, which when the average lengths of the forearms are considered equals 6.5 and 4 centimeters. In a measure this difference is obscured for the flexed arm of the European deflects more than does that of the negro.

The conclusion of this study is that the degree of motion of the elbow is greater in the female than in the male and that the lateral deflection of the hand, from flexion of the elbow to extension is much greater in the negro than in the European. The lateral deflection of the hand in the extended arm is much greater than the artistic ideal.

MEASUREMENTS.

Nearly all of the measurements are from arms taken from individuals belonging to the laboring classes. The American negro is more or less intermixed with European blood; those in Baltimore are, however, usually over three-fourths black.

NEGRO, MALE.

Right Arm.

No.	Length of Ulna.	Angle of axis of elbow joint.		Degree of movement of ulna.		Lateral angles of ulna in different positions		
		Humerus.	Ulna.	Flexion.	Extension.	Maximum Flexion.	Right Angle.	Maximum Extension.
909.	255	86	83	45	162.5	4.5	5.5	11
925.	270	82	97	50	169	-4.5	-1	1
956.	260	85	92	47	162	-8	-5	3
1062.	290	80	84	41.5	173	1.5	8	16
1072.	268	67	100	33	165	-7.5	0	13
1106.	290	76	90	35	176	-1	4	14
1126.	270	87	88	35	175	3	3	5
1129.	275	83	94	43	168	-11	-6.5	3
1136.	265	75	94	43	168	-3.5	3.5	11
1142.	240	84	84	48	180	-3	.5	12
1190.	260	83	87	39.5	181	0	2	10
1163.	260	86	80	38	162	5	7	14
1172.	280	86	84	47.5	163	-1	1	10
1187.	240	79	91	31	167	-2	9	10
1190.	290	85	85	33	166	4	5	10
1230.	245	87	81	42	161	3.5	7.5	12
1261.	275	85	82.5	39	170.5	3	5	12.5
1271.	270	83	85	42	168	0	4	12
1275.	265	82	87	40	167	-8	-2	11
1285.	275	81	94	39	162	-4	0	5
1315.	270	87	81	40	163	5	6	12
—	300	84	86	40	168.5	-3	1	10
—	285	83	85	44	155	-4	2	12
Average (23)	269.5	82.5	87.5	40.5	167.5	-1.3	2.6	10

Left Arm.

925.	280	83	92.5	46	192	3	6	4.5
909.	255	85	86	47	164	6	6	9
956.	270	82	91	44	168	-8	-3.5	7
1062.	285	82	82	38	174	0	5	16
1070.	266	75	94	42	179	-7	0	11
1106.	290	73	89	36	173	3.5	9	18
1123.	250	75	81	38.5	161.5	2.5	6	14
1125.	270	84	85	43	177	5	7	11
1126.	275	84	90	34.5	163	-3	1	6
1128.	290	83	88	40	170.5	6	4	9
1136.	265	78	91	42	172	2	4.5	11
1142.	245	75	94	39	180	-3	1.5	11
1155.	290	87	85	34	175	-2	-1	8
1157*.	234	80	91	36.5	184.5	3	6	9
1187.	240	90	82	45	158	-1.5	0	8
1190.	260	85	84	46	167	1	4	11
1191.	255	79	85	41	184	7.5	11.5	16
1208.	230	83	81	44.5	173	5	8.5	16
1230.	232	83	80	48	164	3	8.5	17
1261.	278	85	80	36	164	6	8	15
1271.	273	85	85	37	167	-2	3	10
1275.	270	83	86	38	173	-1	5	11
1285.	285	86	91	36	166	0	1	3
1315.	260	88	90.5	34.5	163	3	1.5	1.5
—	295	80	84	38.5	173	0	9.5	16
—	255	80	95	39	181	-16	-7	4.5
Average (26)	265	82	87	40	172	.5	4	10.5

*Boy.

NEGRO, FEMALE.

Right Arm.

891.....	270	83	83	46	184	-1	5	14
1120.....	250	85	79	36	175.5	4.5	6.5	16
1141.....	265	80	87	30	171	-2	5.5	13
1176.....	245	85	85	32	177	2.5	2.5	10
1188.....	238	80	88.5	40	180	-1	3.5	11.5
1220.....	240	86	81	31	164	5	9	13
1221.....	240	83	85	38	180	4	8	12
1228.....	240	80	88	39	175	0	5	12
1272.....	240	87	76	37.5	168	7	7	17
1365.....	255	88	76	36.5	193	6	7.5	16
Average (10)....	248	83.5	83	36.5	177	2.5	6	13.5

Left Arm.

891.....	260	79	90.5	43	182	-4.5	0	10.5
1120.....	245	83	77.5	19.5	173	7	11	19.5
1141.....	260	80	92	29	171	-3.5	2.5	8
1166.....	237	85	87	27	170.5	4	4	8
1188.....	235	80	92	35.5	180	-3.5	.5	8
1220.....	243	80	83	29	173	7	13	17
1221.....	240	83	86	41	174	3	6.5	11
1228.....	245	75	94.5	36.5	175	-4	1	10.5
1268.....	255	80	91	30	179	-6.5	0	9
1272.....	240	80	83	37	170.5	3	7	17
1365.....	250	80	85	37	193	8.5	11.5	15
Average (11)....	246	80.5	87.4	33	176.5	1	5.2	12.1

EUROPEAN, MALE.

Right Arm.

983.....	240	83	88	41	172	-1.5	3	9
1123.....	245	83	81.5	45	165.5	6	8.5	15.5
1138.....	235	84	88	43.5	175	-9	-5	8
1140.....	270	85	81	30	169	9	10	14
1146.....	235	90	81	41	177	8	4	9
1258.....	255	85	84	35	167	4.5	4.5	11
1161.....	250	80	85	33	181	0	5	15
1286.....	250	83	87	43	163	2	5	10
1292.....	260	89	84	37	173.5	6	6.5	7
—.....	255	84	89	43	185	0	2.5	7
Average (10)....	249.5	84.5	85	39	173	2.5	4.5	10.5

Left Arm.

1146.....	230	87	89	40.5	173	8	6.5	4
1161.....	255	75	87	57	166	6	9.5	18
1195.....	270	83	84	33	171	7	7	13
1286.....	253	80	93.5	35	172	-2	-1	6.5
1292.....	265	85	84	31.5	169	5	8	11
Average (5)....	255	82	87.5	39.5	170	5	6	10.5

EUROPEAN, FEMALE.

Right Arm.

1131.....	225	77	90	37	183	-2.5	2.5	13
1225.....	225	82	88	44	175	-4	1	10
Average (2)....	225	79.5	89	40.5	179	-3.25	1.75	11.5

Left Arm.

1131.....	220	82	87	40	188	3	6	11
1225.....	223	83	85.5	40	167	.5	7	11.5
Average (2)....	221	82.5	86.5	40	177.5	1.7	6.5	11.3

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A STUDY OF THE LOCATION AND ARRANGEMENT OF THE GIANT CELLS IN THE CORTEX OF THE RIGHT HEMISPHERE OF THE BONNET MONKEY (MACACUS SINICUS).

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WITH 3 FIGURES.

In view of the recent experimental results regarding the representation of movements in the cerebral cortex, a more exact study of the distribution of the large pyramidal cells as seen in microscopic sections has seemed to me desirable. The following paper deals with the distribution in the bonnet monkey.

The right hemisphere of a healthy adult monkey (*Macacus sinicus*) hardened in Müller's fluid, dehydrated and imbedded in celloidin, was cut in horizontal sections 50 microns thick. The sections were numbered from below upward, and stained first by Pal's modification of Weigert's haematoxylin method, counter-stained with carmine and mounted in balsam. This double stain has the advantage of accentuating the contrast between cells and fibers and facilitating the study of the relation of the cells to the various fiber tracts.

A careful study of these serial sections reveals the following arrangement of the Betz (giant) cells in the motor cortex and, if confirmed by the study of their disposition in other specimens, may lead to some modification of the present ideas in relation to the extent of the so-called "motor areas of the cortex.

On the external surface of the hemisphere the lowest point at which any giant cells are found corresponds to the lower extremity of the fissure of Rolando. A few scattered cells are found here upon the anterior lip, but quite in the depth of the fissure. From this point upward they gradually increase in number and at a point about 0.5 mm. higher up there is a small group of giant cells between the corona radiata of the ascending frontal convolution and the surface, but the majority of these cells is still within the fissure contiguous to what may be called the posterior aspect of the corona radiata of the ascending frontal con-

volution. Another small group of giant cells appears in a corresponding position, after an interval of 10 sections (0.5 mm. higher on the surface) contiguous to the external aspect of the corona radiata of the ascending frontal convolution. This arrangement of the giant cells in groups does not obtain within the fissure of Rolando, but they appear here as a continuous layer in gradually increasing numbers from below upward. Nor is there any further appearance of grouping of the giant cells on the external aspect of the corona radiata of the ascending frontal convolution, but from this point upward they extend farther and farther forward until in the level of the anterior limb of the frontal sulcus they cover the entire antero-posterior extent of the external aspect of the corona radiata of the ascending frontal convolution. At a slightly higher level the giant cells entirely envelope this process of the corona radiata; that is, they are present upon its posterior, external and anterior aspects, and as we reach still higher levels extend a short distance forward in contiguity with the external surface of the corona radiata of the frontal lobe (Fig. 3).

This arrangement is maintained throughout the remainder of the upward extension of the corona radiata, which, in the monkey, corresponds to the cortex of the ascending frontal and the posterior portion of the superior frontal convolution (Fig. 1).

This distribution of the giant cells upon the external surface of the brain is by no means uniform. They are most numerous within the sulcus of Rolando and in that portion of the cortex covering the ascending frontal convolution, while in the cortex of the superior frontal they are more scattered. Within the sulcus of Rolando they extend to the base of the sulcus, but are confined entirely to the anterior lip; that is, they nowhere pass beneath the base of the fissure to the parietal lip.

There are two small groups of large cells in the cortex of the ascending parietal convolution: one just above the lower extremity of the intra-parietal fissure appearing in fourteen consecutive sections, the cells diminishing in number and size from below upward. These cells extend into the intra-parietal fissure but not into the fissure of Rolando. There is another small group of large cells in the cortex of the upper extremity of the ascending parietal convolution, only present in four sections. With the exception of a few very large cells within the intra-parietal fissure, the cells described in the cortex of the ascending parietal convolution are much smaller than the majority of the giant cells anterior to the fissure of Rolando. Those anterior to Rolando measure from 20 to 60 microns in length by from 10 to 40 microns in

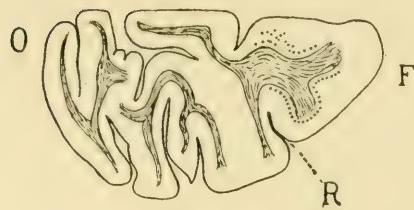


FIG. 3.

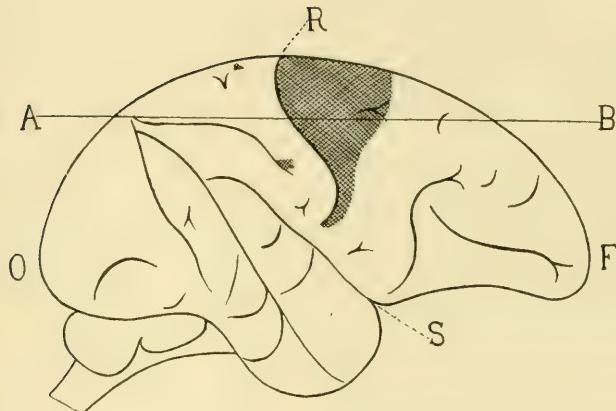


FIG. 1.

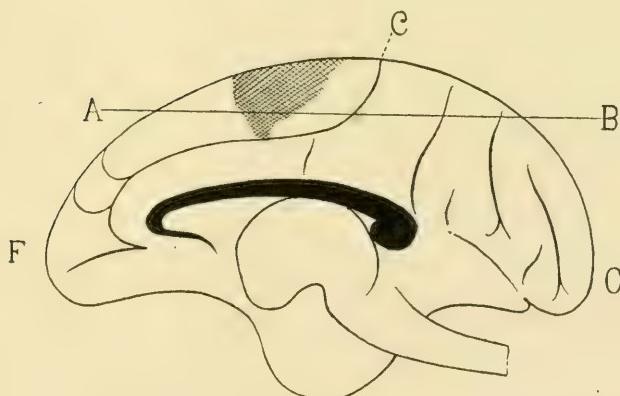


FIG. 2.

DISTRIBUTION OF GIANT CELLS (BETZ) IN CORTEX OF BRAIN (*MELLUS*).

breadth, while those in the cortex of the ascending parietal convolution, with the exception noted, are from 15 to 22 microns long by 12 to 18 microns wide.

On the mesial surface of the brain the superior border of the cortical area in which the giant cells are found corresponds exactly with the superior border of that upon the external surface; that is, the anterior and posterior borders of this area may be followed directly over from the external convex surface of the brain upon the mesial surface. The area occupied on the mesial surface is a somewhat irregular triangle with its apex corresponding to the upper extremity of the fissure of Rolando and its base directed toward the frontal pole. The giant cells are relatively more numerous upon the mesial surface. Instead of being arranged in a single layer, as upon the external surface, they are more irregularly scattered about in groups of several superimposed layers. Cells of the larger diameters are also relatively more numerous on the mesial than on the external surface. The area extends downward to the calloso-marginal sulcus about 8 mm. below the crest of the hemisphere. The cells in the lower portion of this area are less numerous and rather smaller than elsewhere on the mesial surface.

EXPLANATION OF FIGURES.

Note that, for the sake of comparison, Fig. 3 is placed above Fig. 1.

FIG. 1. External surface of right hemisphere; *R.* Fissure of Rolando; *S.* Fissure of Sylvius; *F.* Frontal pole; *O.* Occipital pole; *AB.* Plane of section of Fig. 3. Area of distribution of giant cells is striated.

FIG. 2. Mesial surface of right hemisphere. *C.* Calloso-Marginal sulcus; *F.* Frontal pole; *O.* Occipital pole; *AB.* Plane of section of Fig. 3. Area of distribution of giant cells is striated.

FIG. 3. Horizontal section at line *AB*, Figs. 1 and 2, showing arrangement of giant cells at that level. *R.* Fissure of Rolando; *F.* Frontal pole; *O.* Occipital pole.

A THREE WEEKS' HUMAN EMBRYO, WITH ESPECIAL
REFERENCE TO THE BRAIN AND THE NEPHRIC
SYSTEM.

BY

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WITH 5 PLATES.

The specimen under consideration was loaned to me sometime ago by Dr. Mall for the investigation of the evidence of segmentation in the brain of the higher mammals.

While the study of this embryo is of necessity incomplete in the present state of knowledge, certain points were shown so clearly by it, that they are here presented.

These points are:—For the central nervous system (a) the approximate location of the morphologic front of the brain, and (b) the location of folds and lobules of the fore- and hind-brain which may have segmental significance; for the nephric system, (a) the presence of a pronephros and (b) a generalized state of the mesonephros, illustrating well-known conditions in the lower as well as in the higher forms.

Other facts which agree with or differ slightly from those generally accepted are presented incidentally.

The specimen is No. 148 of the Johns Hopkins University Medical School Collection gathered by Dr. Mall, sectioned under his direction, and most generously opened for inspection to students. In the catalogue of this collection¹ is found a list of nine papers which use this specimen to illustrate special points. These will be referred to under the proper headings.

The embryologic collections of Cornell University and of the Harvard and Johns Hopkins Medical Schools were also freely placed at my command for study and comparison.

¹ Mall, F. P., Johns Hopkins Hosp. Bull., XIV, 1903. Catalogue of the collection of human embryos in the Anatomical Laboratory of the Johns Hopkins University. Baltimore, 1904.

MODELS AND DRAWINGS.

A model of the entire specimen was made by the Born method,² modified as described by Bardeen.³

As the sections were not equally perfect, the best of each group of three was drawn at a magnification of 66 $\frac{2}{3}$. The sections are 10 μ thick, and as but one-third of them was drawn, each wax plate of which the model was made, represents the thickness of three sections, or 30 μ . The reconstruction was laid out on an outline enlarged from the photograph made by Dr. Mall.⁴ The completed model has a length of nearly 300 mm. A comparison of the model with the photograph taken before embedding shows that in the process, there had been a shrinkage of 10 to 12%.

The model was used as a basis for the drawings, the contour line of every fifteenth or thirtieth section being indicated. In this way the different levels can be correlated throughout the set of drawings which represent the model sectioned at different planes. The drawings in each case, were corrected by repeated comparison with the specimen.

The form of the celom was obtained by building up the parts cut from the entire model.

Models at a much greater magnification were made of various details, as the fore-brain, the mesonephros and mesonephric tubules.

A part of the details are illustrated by reproductions of photographs of sections of the embryo.

EXTERNAL FORM.

This embryo was photographed by Dr. Mall at three times enlargement. The photograph, published in an article by him,⁴ shows the specimen from the right side, lying upon the opened chorion and is described as "An embryo three weeks old." He also mentions that the umbilicus is at the right instead of the left, as is the more usual position. In another paper, he shows an enlarged outline drawing.⁵

The general form is shown in Figs. 1 and 1a, the body being about once and a half as long as the head and at an angle of about 65° with it. The face is featureless except for the wide slit-like mouth (Fig. 2). At the corner of the mouth (Figs. 2, 5, 6) is a depression with a thin gill-cleft-like membrane. The four gill-clefts are irregularly spaced giving the

² Born, G., Morph. Jahrb., II, 1876; Arch. f. mikr. Anat., XXII, 1883.

³ Bardeen, C. R., Johns Hopkins Hosp. Bull., XII, 1901.

⁴ Mall, F. P., Welch Anniversary Volume, 1900; also Johns Hopkins Hosp. Reports, IX, 1900.

⁵ Mall, F. P., Johns Hopkins Hosp. Bull., XII, 1901.

second arch great prominence while the third and fourth are in a common depression, the sinus precervicalis. On the right the gill-clefts are less crowded and the sinus not quite so deep. The first gill-cleft is wider at its dorsal end indicating probably a beginning of the external meatus.

The heart is prominent. The yolk sac is extensive turning to the right instead of the left as is more usual, thus making the umbilicus more apparent in a view from the right than from the left side (Figs. 5, 6).

The limb-buds are remarkably prominent in comparison with other specimens supposed to be of the same age.

Epithelial thickenings.—In Fig. 1 are mapped out the regions having a thickened epithelium. Details of these thickenings as shown by individual sections are found in many of the figures, as the arm (Fig. 10, 11), the leg (Fig. 5), anal plate (Fig. 1), gill-arches (Figs. 1, 11), mouth (Fig. 5), olfactory region (Fig. 2), lens (Figs. 1, 2, 5, 8, 16), and the neuropore (Figs. 1-8, 16). In Fig. 1 the thickness of the epithelium corresponds with the density of the dots. The portions left white represent one layer of cells which become flattened over the heart and near the dorsomesal line. Over the entire oblongata this layer of flattened epithelium coalesces with the wide, thin roof of the brain (Fig. 4). Mall⁵ calls attention to the thickening of the neuropore in this specimen. Especially noticeable is the H-shape of the thickening over the olfactory and cerebral regions (Fig. 2). The continuity of leg-bud thickening with that of the anal plate (Fig. 1) is comparable to the condition in amphibian embryos.

AGE OF THE SPECIMEN.

The exact age of this specimen cannot be determined any more closely than has been done by Dr. Mall, who considers that it is of about twenty-one days development. Its relative age is somewhat important since it presents certain features not hitherto fully described. It is necessary to determine whether it may be a transitional stage or a pathologic or arrested condition which is under observation.

Though it presents one feature (the small number of thoracic myotomes) not universal, and others (the umbilicus turning to the right) which are not common, and still others rare or not previously observed, still on the whole, it so well fits into the series which has been described by various authorities or examined by the author that the weight of evidence seems to point in the direction that this is in general a normal transitional condition, though possibly somewhat exaggerated in a few particulars or retarded in others.

Comparing this specimen with the His models and Atlas,⁶ it is seen that there are some resemblances to his specimen *Lr* estimated at twenty-one days, but in external appearance, it seems to lie between *a* and *R* estimated by him to be from 21 to 25 days.

The specimens No. 164, 209, 148, and 80 in the Johns Hopkins collection show that in certain features of development they form a progressive series (see Nephric System, below). The formula devised by Mall,⁷ for determining the age ($\text{days} = \sqrt{100} \times \text{length of embryo}$), approximately worked out gives the following:

TABLE I.

No.....	209	164	148	80
Length in mm.....	3 +	3.5	4.3	5 or 4.5
Myotomes.....		19	28 to 29	
Days	17 +	19 —	21 —	22 +

THE ALIMENTARY CANAL AND ITS APPENDAGES.

Mouth.—The mouth (Figs. 1-4) is simply a cleft between the fore-brain region and the mandible, extending laterally to the just forming maxilla and at its tips having a thin membranous portion (Fig. 5) in section strongly resembling the membranous tips of the gill-pouches.

No remnant of the oral plate was found. The position of the dorsal limit of the ectodermic portion of the oral plate is indicated by the hypophysis (Figs. 9, 3, 4). The latter is a bi-lobed, widely opened pouch in contact with the infundibulum of the brain and partially surrounding it.

Pharynx.—No signs of Sessel's pocket, the most cephalic of the entodermic structures of the pharynx could be found since the sections are not favorable in this region.

The lateral or lingual folds, described by Kallius⁸ as forming the first rudiment of the tongue, are represented in the mesal view (Fig. 4), as a ridge lying at the side of a mesal pit. The *tuberculum impar* has not yet arisen in front of the median thyroid. The latter body with the pit from which it arises is present, but the tubular connection between gland and pit has nearly disappeared (Figs. 3, 4).

The floor of the pharynx is partially exposed in Fig. 11, showing lateral

⁶ His, W., *Anatomie Menschlicher Embryonen. Text and Atlas.* Leipzig, 1880-1885.

⁷ Mall, F. P., *Age of Human Embryos.* Ref. Handb. Med. Sci., 2d Ed., III, 1901.

⁸ Kallius, E., *Anat. Gesell., Verhand,* 1903.

extensions into the four gill pouches, each of which ends in a thin plate. The location of the membranous tips of the gill pouches is indicated upon the mesal view (Fig. 4). The gill pouches have also small ventrally projecting blind pouches ending in a somewhat thickened epithelium, beginnings or protots of the thymus and lateral thyroid bodies.

The larynx is represented by a slight depression, on the ventro-lateral borders of which is a pair of minute pouches (Fig. 11) similar in appearance to the ventral processes from the gill pouches. From their general relations it seems probable that they represent the rudiments of a 5th pair of gill pouches.

A tubular projection in the roof of the pharynx over the entrance to the esophagus is apparent in several sections. Killian⁹ identified a mesal pouch occupying a position just caudal of the pharyngeal tonsils as the *Bursa pharyngea* of Meyer. He traced this back to the 11th week of embryonic development. The specimens of the 7th to the 12th week in Cornell University make it apparent that the pouch seen in Figs. 3, 4, at the left of the abbreviation *ch.* is this same *Bursa*.

Trachea.—The trachea (half a mm. long) ends in a pair of widely-spreading bronchi, each with a single slightly enlarged end, the lung-bud (Figs. 3, 11), surrounded by an enlargement in the mesentery.

Alimentary Canal.—The esophagus is small and practically closed through part of its length. It extends to section 107, where it merges gradually into the stomach which shows a spindle-shaped enlargement increasing at its caudal end and turning to the left (Figs. 3, 11). The lesser peritoneal cavity pushing the stomach to the left (Figs. 2, 17) is shown at its opening into the cœlom (Fig. 11, crossed by line pointing to mesentery).

The stomach narrows again as it merges into the duodenum (Fig. 10). A minute dorsal enlargement of the duodenum is the rudiment of the pancreas (Fig. 3). On the ventral side is found the short bile duct (Figs. 3, 6). As somewhat diagrammatically shown in Figs. 6 and 10, the trabeculae of the liver are in a great sinusoid along the path of the vitelline vein. In Fig. 11 both lobes of the liver are shown from the dorsal side, and in Fig. 6 there is a section through it at the level of the bile duct. The duodenum is enlarged at this point of union with the bile duct, and continues as a tube to its wide (260μ) union with the vitelline sac (Fig. 5).

The caudal intestine within its free dorsal mesentery (Fig. 5) continues from the vitelline sac in a curve following the back. It enlarges

⁹ Killian, G., Morph. Jahrbuch, XIV, 1888.

again as it leaves the free mesentery and curves around the end of the cœlom, and unites with the allantois to form the wide cloaca which is joined by the Wolffian duct (Figs. 5, 17).

The cloaca is closed by the anal membrane, a thickened plate with only a slight indentation (Fig. 17).

Allantois.—The allantois extends as a narrow tube along the abdominal stalk and bending over the caudal end of the cœlom (Fig. 5), enlarges to form the bladder as it unites with the cloaca (Fig. 17).

From the standpoint of the development of the diaphragm Mall¹⁰ gives the following description of the organs of the thoracic and abdominal cavities:—"Sections of the embryo 4.3 mm. long (No. 148) show the liver sprouts growing in all directions through the septum transversum, encircling and ramifying through the omphalo-mesenteric veins, making a condition slightly in advance of that in His's embryo *Lr*. The sections of this embryo show clearly, that the heart, lungs, liver, and lower peritoneal cavity arise in tissues surrounded by that portion of the cœlom extending into the head in Embryo XII. . . . The lungs arise when the pericardial cœlom goes over into the pleural, *i. e.*, high up in the region of the head. Immediately on the dorsal side of them is the beginning of the lesser peritoneal cavity, and the intestinal tube struck in this section is the stomach. All these structures lie on the cephalic side of the first cervical myotome. Projecting into the peritoneal cœlom, encircling and penetrating the omphalo-mesenteric veins, are the projections of the liver. The two lobes reach from the tip of the lungs and the foramen of Winslow to the point where the entodermal cells of the liver arise from the alimentary canal, or in this case, the duodenum. The lobes of the liver lie entirely within the canals of the cœlom on either side of the head. The caudal ends of these cœlom canals have migrated from opposite the second cervical myotome in Embryo XII, to opposite the second thoracic myotome in Embryo 148. It has moved toward the tail, while the cephalic end of the canal, the pericardial cœlom, has been kinked over to correspond with the growth of the heart. . . . We have in the embryo the necessary stage to locate the organs which arise in the neighborhood of the septum transversum, as well as to give the fate of the cœlom in their immediate neighborhood."

The points of the above description illustrated by the figures are: the penetration of the septum transversum by liver substance and blood-vessels (Fig. 10); the continuity of pericardial and abdominal cœlom (Figs. 2, 11); the position of lung, stomach, liver, and it may also be

¹⁰ Mall, F. P., Johns Hopkins Hosp. Bull., XII, 1901.

said, duodenum, in the coelom canals which lie dorsad of the septum transversum (Fig. 3); the caudal end of the coelom canals opposite the 2d or perhaps the 3d thoracic myotome (13th myotome).

It seems to me that the most cephalic portion of the pericardial coelom might be said to be that surrounding the bulbus arteriosus, in which case it would be difficult to decide that it had retreated so far from its original position opposite the ear as Mall has mentioned.

The specimen and the model show more clearly than any of the drawings, the caudal traction of the organs which has taken place while their original cephalic attachment can still be traced. The furrows of the mesentery (Figs. 3, 6, 10, 11) show this more clearly than the varying caliber which indicates the division of the entodermal tube into organs. The furrow, for instance, separating duodenum and yolk sac extends cephalad to the 8th myotome (6th cervical), and the attachments of all the entodermal organs cephalad of it are spread out between this point and the 1st occipital myotome, that is in the neck. From this, it hardly seems correct to say that lung and stomach are really so far cephalad as mentioned by Mall (*i. e.* in the head).

MESODERM.

General.—The general appearance of the mesodermic tissue is shown in Plate V, being entirely cellular. The condensations in the nephric region are described below. Other condensation is seen in the limbs, but without clear differentiation into separate masses. Condensation also occurs at the side of the pharynx and markedly so in the floor. This continues uninterruptedly into the thick mesentery surrounding lungs, esophagus, stomach, and intestines (Figs. 2, 3, 6, 10, 11), and in the region of the cloaca joins the condensation around the Wolffian duct and continues into and fills the leg-bud.

A distinct spindle-shaped condensation of cells occurs ventrad of the eye (Figs. 7, 8).

Myotomes.—Bardeen and Lewis¹¹ give the following description: "Length, neck-breach, 4.3 mm.; age about three weeks. . . . Though more advanced in development than *Lr* (His), but twenty-seven myotomes are present (2o, 8c, 10t, 5l, 2s). This has been determined by careful counting of the myotomes in serial sections of the embryo. The base of the arm-bud appears to lie opposite the seventh to the eleventh myotomes. It is, therefore, probable that two occipital myotomes are present. But nine myotomes lie in the area between the arm-bud and the

¹¹ Bardeen, C. R., and Lewis, W. H., Amer. Jour. Anat., I, 1901.

leg-bud. The base of the latter lies opposite the 21st to the 25th or 26th myotomes. If two myotomes be considered occipital myotomes, the leg, in this instance, lies two segments nearer the head than usual. It is therefore probable that this embryo has an unusually short body-wall."

Twenty-nine myotomes were counted and modelled on the left side, and twenty-eight on the right, the discrepancy occurring in the caudal region. This count in general, agrees with Bardeen and Lewis; 2 occipital, 8 cervical, 10 thoracic, 5 lumbar, and 3 or 4 instead of 2 sacral, as noted by them.

The arm-pads have even a longer cephalo-caudal enlargement than noted by Bardeen and Lewis, and cover the 7th to the 13th myotomes, thus leaving only 7 complete myotomes between the arm- and leg-buds.

Many of the myotomes do not model in the regular forms usually shown. The first occipitals are small and imperfect (Figs. 14, 15). The 3d, 4th, and 5th are dorsally composed of two distinct, hollow horns (Fig. 14), which merge ventrally into a common cavity. More ventrally they become solid and are marked across the middle by a band of cells (Fig. 15, at left). The lumen is not large until the 11th (Fig. 11), from which point until near the end of the series it is a marked feature. The largest and most regular myotomes are opposite the legs, the most irregular among the cervical.

In several myotomes (Figs. 5, 11), careful examination could detect no limitations between a certain area at their ventral end and the condensed mesoderm of the limb-bud, in fact in these cases, the appearance would indicate the origin of limb tissue from myotomes.

Evidences of segmentation in the mesoderm are also seen cephalad of the two clearly recognizable, occipital myotomes 1 and 2, and immediately in line with them. Cephalad of the 1st is a minute area with apparently the identical structure of a myotome, including the familiar corrugation of the epidermis (Fig. 14). Still more cephalad are two other lesser condensations and corresponding epidermal corrugations (Fig. 15). That is, there are indications of three more occipital myotomes than are distinctly figured.

Sclerotomes.—On their mesal aspect the mesodermic tissue is shrunken away from the myotomes in loops (Fig. 15), and the tissue shows a condensation (sclerotome) corresponding with each loop down to the 18th myotome. In the cephalic region a slight condensation occurs ventrad of the notochord. That is, these last two points indicate a slight differentiation and segmentation of sclerotogenous tissue. In some sections, the continuity of these sclerotomes with the myotomes can be seen.

Dermatomes.—This specimen by itself does not throw much light on the question as to the fate of the outer wall of the myotome, *i. e.*, whether it is in reality a dermatome or not.

VASCULAR SYSTEM.

Heart.—In Figs. 1, 3, and 5 the position of the heart is seen, and in Fig 12, a ventral view showing a compact, somewhat square form, reminding one of the His' model from a 10 mm. (4 week) specimen rather than those from younger specimens. The greater length of the right auricular portion is similar to the His model of a 5 weeks' embryo. A very young embryo, modeled and presented by J. L. Bremer¹² at the American Association of Anatomists, in December, 1904, shows an exaggeratedly long right auricle. In external form, the heart of this specimen resembles older, rather than younger stages, but the internal relations accord with the descriptions given for the 3 weeks' stage. That is, the tubular heart is already dividing into right and left (Figs. 2, 6); the entrance of the sinus venosus is decidedly to the right (Fig. 2, left of Fig.), the connection of the auricle with the ventricle is through a narrowed tube, the auriculo-ventricular canal (Fig. 10), entering the ventricle at the left (Fig. 6), while the bulbus arteriosus makes its exit at the right (Figs. 12, 6), turns sharply cephalad and extends along the ventral aspect of the heart.

The walls of the heart contain only undifferentiated muscular tissue. In parts the endothelial tube is closely applied to the walls; in parts, notably along the path of the auriculo-ventricular canal (Fig. 10), the ventral end of the right ventricle (Fig. 6) and of the entire bulbus (Figs. 10, 2), the endothelial tube is connected with the outer wall by only a delicate mesh-work of tissue.

Arteries.—The endothelial tube of the bulbus plunges into the floor of the pharynx, expands into a wide sinus, giving off on each side near the middle line, a small branch which divides into the 1st and 2d gill arches and a mesh-work of capillaries supplying the mandible. The 1st and 2d arches join with the dorsal aorta by a very slender capillary connection. The 3d and 4th gill arches (Fig. 11) are given off from the side of the sinus and unite dorsally to form the main portion of the dorsal aorta. The 5th gill arch is given off caudally near the middle line, divides into capillary branches, supplying the larynx and trachea (5th and 6th arches), reaching the dorsal aorta by very small branches. Each dorsal aorta sends forward a branch which can be traced in the roof of the

¹² Bremer, J. L., Amer. Jour. Anat., IV, No. 2, p. VIII.

pharynx nearly to the hypophysis. This portion of the vascular system resembles closely others described of the same age, but reminds one, in the extreme difference in size of the aortic arches, of the condition described by Miss Lehmann,¹³ where in lower mammals the six arches are not all complete at any one time.

The two dorsal aortæ unite (Fig. 3) near the cephalic border of the liver and the caudal margin of the arms, giving off just before their union the brachial arteries and soon after, the small vitelline or omphalo-mesenteric artery (Figs. 10, 17). Branches could occasionally be traced to the tubules of the mesonephros (Fig. 6). In contrast to the other arteries the umbilicals are large and in the body stalk anastomose across the middle (Fig. 5), but continue as a pair for some distance in the body stalk.

Veins.—The veins are remarkable for the great variation in caliber. The much branched jugular (precordial) can be traced from a point lying between the eyes and cerebrum (Fig. 7), keeping near the surface (Fig. 8), sending branches through, and then passing mesad of the Gasserian ganglion (Fig. 9), laterad of the ear vesicle and the ganglia of the 7th and 8th nerves (Fig. 11), and by a breaking-up in the 9th and 10th ganglia, comes to lie mesad of the 10th ganglion as it passes over into the vagus nerve, then unites with the cardinal (Fig. 11, at left) and the umbilicals (Fig. 2, at left) to form the ducts of Cuvier. The course of the jugular in the head seems to illustrate one phase of the change of position of the bloodvessels with relation to the nerves as demonstrated by Dr. Mall¹⁴ in a recent article.

The ducts of Cuvier unite across the middle to form the sinus venosus; and this connects by a small opening with the great sinusoid¹⁵ of the liver formed in the course of and by the union of the vitelline veins (omphalo-mesenteric) (Fig. 10).

The umbilical veins coming from the body stalk are joined by veins from the legs, become enlarged and break up in the body wall into a great sinus (Figs. 5, 6, 10, 11), and finally as they enter the duct of Cuvier become so small as to be scarcely traceable (Fig. 2, at left, and Fig. 10, at right).

THE NEPHRIC SYSTEM.

General.—The nephric system (Fig. 17), as is usual at this age, consists of a pair of Wolffian ridges extending along the back from the arm-

¹³ Lehmann, Harriet, *Anat. Anz.*, XXVI, 1905.

¹⁴ Mall, F. P., *Amer. Jour. Anat.*, IV, 1904.

¹⁵ Minot, C., *Boston Soc. Nat. Hist., Proc.* XXIX, 1900.

pad nearly through the region of the leg-pad, and of Wolffian ducts opening into the cloaca. Sections of the ridges and their contained structures are shown at different levels, beginning cephalad, in Figs. 11, 18, 10, 19, 6, 20, and 5.

There is no definite thickening in the coelomic epithelium or the mesoderm which can be designated as a rudiment of the genital ridge or of the Müllerian duct.

In the ridges, the Wolffian duct is traceable as indicated by dots in Fig. 17 from the 1st mesonephric tubule along the lateral border of the ridge and extending beyond the coelom in a curve (Fig. 5), to open into the lateral border of the cloaca (Fig. 17). Extending still farther laterad along the Wolffian ridge is the cardinal vein, the diameter of which varies greatly in different sections (Figs. 18, 19).

Adrenal.—Near the cephalic end of the Wolffian ridge (Figs. 17, 11, suprarenal), on the mesenteric border are slight folds in the epithelium which remind one of the structures in the cephalic part of the mesonephros found by Aichel¹⁶ in the rabbit to be associated with the development of the adrenal or suprarenal body.

Pronephros.—A wide open funnel (Figs. 17, 18), opening to the coelom and connecting with a small, blind tube extending cephalad for a few sections, is here called a pronephric tubule on account of its position in the cephalic portion of the Wolffian ridge. It has no connection whatever with the Wolffian duct and is separated by a marked interval from the beginning of that duct. This is apparently only a further retrogression from the condition found by MacCallum¹⁷ in which a separated portion of duct extends opposite the 6th, 7th, and 8th myotomes of an embryo 3.5 mm. long. MacCallum calls attention to the possibility that this separated tube with its cephalic opening through a funnel to the coelom may be a pronephric remnant. In an older embryo (5 mm. long) he finds such a disconnected remnant with a single tubule not opening to the coelom.

Tandler¹⁸ says that in human embryos from 5 to 20 mm. he found pronephric tubules in communication with the coelom eight times. These were at the level of the 5th to 6th segment.

In Embryo 148, the isolated pronephric tubule lies opposite the 11th myotome. Whether the difference in position relative to the myotomes from those reported by MacCallum and Tandler, indicates that this is not

¹⁶ Aichel, Otto, *Arch. f. mikr. Anat.*, LVI, 1900.

¹⁷ MacCallum, J. B., *Amer. Jour. Anat.*, I, 1902.

¹⁸ Tandler, J., *Centralbl. f. Physiol.*, XVIII, 1904.

a true pronephric remnant, or whether a caudal shifting of the Wolffian ridges has taken place, or whether there is an increase in the number of myotomes in the occipito-cervical region of No. 148 cannot be determined. But at present, it is unmistakable that in three embryos of $2\frac{1}{2}$ to 3 weeks in development (Nos. 164, 148, and 80 of the Mall collection) there exists a structure which has many resemblances to a pronephros.

In a 3 mm. human embryo, Janosik¹⁹ found that there is a distinct glomerulus protruding into the cœlomic cavity. The position of this glomerulus is similar to the tubules found in the embryos of the Mall collection.

Tandler²⁰ found one glomerulus in eight cases. It seems, therefore, that in spite of the apparent discrepancy of position, there should be no hesitation in expressing the homology of the structure under consideration with the pronephros of lower forms.

Mesonephros.—A preliminary report on the generalized constitution of the mesonephros of Embryo No. 148 was presented with models at the December, 1903, meeting of the American Anatomists.²¹ The main facts noted were, that in the Wolffian ridges an open pronephric tubule occurs on each side followed by two groups of mesonephric tubules, the first group of eight being of the usual embryonic type, S-shaped, with Bowman's capsule, rudimentary glomerulus, and union with the Wolffian duct; the second group of eleven or twelve, consisting of tubules, none of them uniting with the Wolffian duct and varying from solid aggregations of cells to hollow vesicles and finally to vesicles open to the cœlom. The two sides present the same general arrangement although the tubules do not form symmetrical pairs in precisely the same stage of development (cf. Figs. 17-19).

McMurrich²² says, page 391, "One of the characteristics of the mammalian mesonephros is that it possesses no nephrostomes." Minot²³ in his *Embryology*, page 237, says, "In all amniota the nephrostomes all become completely separated from both the myotomes and peritoneum throughout the region of the Wolffian body, except that possibly in a few anterior segments, the connection with the peritoneum is retained as is suggested by Sedgwick's observations (in birds)."

In an embryo 4.25 mm. in length, Meyer²⁴ showed that in the cephalic

¹⁹ Janosik, J., *Arch. f. mikr. Anat.*, XXX, 1887.

²⁰ Gage, Susanna P., *Amer. Jour. Anat.*, III, 1904, p. VI.

²¹ McMurrich, J. P., *The Development of the Human Body*. Phila., 1902.

²² Minot, C. S., *Human Embryology*. New York, 1892.

²³ Meyer, H., *Arch. f. mikr. Anat.*, XXXVI, 1890.

half of the Wolffian ridge, certain of the tubules are directly continuous with the epithelium covering the ridge, but there are no hollow tubules opening into the coelom. In the caudal half the unsegmented blastema which he considers the fore-runner of the tubules, was not connected with the epithelium. From the description, this is probably a less advanced specimen than No. 148.^{22a}

Janosik¹⁹ found in an embryo, 3 mm. long when fresh, that in the cephalic part of the Wolffian ridge there are a Wolffian duct and a number of independent tubules; the concentrated blastema serially following the tubules, is not segmented, but connects with the coelomic epithelium at intervals.

MacCallum,¹⁷ the latest special investigator on the subject, shows in a 3.5 mm. embryo (164 of the Mall collection) that extending from the 10th to the 19th or last formed myotome there are thirteen enlargements of the Wolffian duct, the 5th to the 8th being considerably elongated, but showing no glomeruli or Bowman's capsules. In No. 80 of the same collection (4.5 to 5 mm. long), there are 17 to 18 tubules with the characteristic S-shaped curve, enlarged Bowman's capsule, and union with the Wolffian duct. He showed in this case a close connection of the Wolffian duct at intervals with the coelomic epithelium. In none of these did the tubules open to the coelom.

It has been my privilege to look over the two specimens described by MacCallum and also a more recent specimen of the same collection (No. 209). These with No. 148 form a series which may throw light on the true development. The following apparently consecutive history is drawn from a study of these embryos ranging from the 17th to about the 23d day (see above, Age of Specimen), and from comparison with various embryos of the Cornell University collection as cat, shark, lamprey, etc.

In the mesonephric region of the least developed stage (No. 164), in respect to the nephric region, the Wolffian duct lying at the dorso-lateral part of the Wolffian ridge has enlargements, some of them, the 5th to the 7th, quite pronouncedly thorn-like. In the next stage (No. 209) in which the sections were thick and details difficult to determine there exists a series of about fourteen rounded tubules with thickened epithelial walls, and a lumen well-defined or in process of formation. In the ventral most prominent part of the Wolffian ridge some of these, as the 5th, are connected by a solid out-growth with the coelomic epithelium of the most

^{22a} Meyer's specimen was 2 mm. long after being hardened, thus making the discrepancy in length less apparent than would be indicated from the measurement 4.25 mm., taken while fresh.

protruding part of the ridge. At the other extremity of the tubule it joins the Wolffian duct.

In a cat from which a part of the mesonephros was modeled, the next stage apparently was found. Some of the centrally located sub-cylindrical tubules open from the cœlom at the crest of the Wolffian ridge, and at the other end of the tubule into the Wolffian duct. The general appearance of these tubules and their connections is perfectly comparable to the condition found in the early shark, the tubules of which were also modeled and found to differ from those of the cat in the fact that they were more S-shaped.

No. 148 (Figs. 17-20) furnishes the next stage. It has apparently in its series of twenty or more tubules a complete recapitulation of the preceding stages co-existing with the commonly recognized type-form of tubule.

There are:—1st, The Wolffian duct in the caudal part, having no connection with the tubules, but presenting a series of distinct enlargements; 2d, Aggregations of cells with no apparent lumen, and no connection with the cœlomic epithelium or the duct; 3d, Cavities or vesicles deep in the Wolffian ridge surrounded by a several-layered epithelium, and having no connection with either cœlomic epithelium or duct; 4th, Similar vesicles lying near to the cœlomic epithelium of the crest of the Wolffian body, connected with that epithelium by (a) a solid string, (b) a narrow open channel, or (c) a wide open funnel (Fig. 20); 5th, In the cephalic half, one tubule, the 7th, with a slight opening from the Bowman's capsule to the cœlomic epithelium; 6th, The remainder (8th, 6th, 5th, 4th, 3d) of the cephalic group have the typical Bowman's capsule, the S-shaped tube, and the connection with the Wolffian duct but no connection with the cœlom (Fig. 19); 7th, The 2d tubule having two Bowman's capsules, one dorsal of the other, that is, an apparent beginning of the dorso-ventral division of the tubules; 8th, The 1st small tubule, apparently consisting only of a Bowman's capsule, sessile on the beginning of the Wolffian duct.

The artery and vein supplying the rudimentary glomerulus of these Bowman's capsules (Fig. 6) could in a few places be clearly seen. The specimen not being favorable to the study of blood-vessels, the vascular supply could not always be made out.

Such a tubule as was found in the cat with both ends open (see above) did not occur in No. 148. The tubules which had attained connection with the duct had in this specimen apparently lost connection with the cœlomic epithelium. In No. 80 (MacCallum¹⁷) the transitional forms

of tubules described above are all lost. The 17 to 18 tubules are complete and like Fig. 19.

In brief, there is, in human embryos, of the 17th to the 23d day, the history of the origin of the tubules from a cellular blastema. This blastema segments into rounded masses, these masses develop cavities that first unite with the coelomic epithelium, then losing this connection, unite with the Wolffian duct which already had definite enlargements, and then finally each tubule forms the thin-walled Bowman's capsule with its glomerulus.

The connection which probably existed between the myotomes and the coelom through the intermediate cell mass which gives rise to the blastema forming the tubules, is of an earlier stage than these here considered. Dr. Minot in the discussion of this paper when presented at the meeting of the American Anatomists stated that in a very early rabbit in the Harvard University Medical School collection, such connection can actually be seen between myotome and nephrotome to the coelom. I have recently seen the same in the chick.

Metanephros.—If any trace of the metanephros or true kidney exists in specimen No. 148, it consists merely of a slightly condensed portion of the blastema, caudad of the mesonephric region where the Wolffian duct extends beyond the coelom (Fig. 17).

CENTRAL NERVOUS SYSTEM.

General.—Most of the figures illustrate some features of the central nervous system. The general outline follows closely the profile shown in Fig. 1. The lateral and mesal views are seen in Figs. 3, 4, and with segments of the model and photographs of the sections together give better than words, the idea of shape. The form more nearly approaches that of embryo *Lr* (4.2 mm. long, with 32 myotomes) shown in His's⁶ atlas (Plate IX, Fig. 13), and the model of the same than any other specimen figured.

The stage of development of eye, olfactory pit, and ear vesicle also put it in the same class, that is, the nervous system is like, in general features, this well-known specimen of three weeks. Details which bear upon the object of the present investigation are not shown by His nor, as far as I know, by other writers on mammalian brains. Mall⁵ in an early presentation of this same embryo mentions the neuropore, which in the present article is fully illustrated and in a way made the starting point for definite conclusions.

The evidence from human and mammalian as well as immammalian

material has been accumulating in the form of specimens, models, drawings, and notes with preliminary papers,^{29 30} until it seems clear that the statements presented with regard to the central nervous system of this embryo are not artifacts due to shrinkage, nor abnormalities, but that the individual characteristics of this specimen may be depended upon to represent one phase of development. This phase is probably transitory because nothing exactly like the neuropore in this specimen has been found in any other specimen examined, though stages both older and younger are seen. On account of the clear demonstration of important facts in transitional stages it seems worth while to record them fully.

The cephalic end of the brain tube and its relation to a serial order of parts.—von Baer² in 1828 represented as perfectly obvious the original cephalic end of the body, including the neural plate, at the point where the hypophysis arises. By more refined methods, Keibel²⁵ in 1889 showed with apparent conclusiveness, that in the rabbit the neural plate, the enteron, and the notochord end in an undifferentiated mass of tissue which is the true cephalic end of the body, and corresponds with the point indicated by von Baer.

From another view point, the place of final closure of the neuropore has been considered by some a crucial test for determining the end of the brain tube. His²⁶ found it at the optic recess and found also that just before final closing, the neuropore is a slit, includes the recessus infundibuli, chiasma, recessus opticus and the olfactory lobes, and extends to the dorsal end of the lamina terminalis. That is, in fact, His really agrees with von Baer and Keibel as to the original condition of the cleft between the neural plates.

Kupffer,²⁷ in sharks and some other fishes, found that the final closure of the neural tube occurs between the olfactory lobes at a point which he calls the *lobus olfactorius impar*. Herrick²⁸ in discussing these widely divergent views concluded that the final closure had no necessary relation to the morphologic front of the brain but that the recessus infundibuli is the primitive cephalic end. Herrick, therefore, also goes back to von Baer's original location of this point.

Accepting then the von Baer-Keibel observations as correct, I

²⁴ von Baer, K. E., Ueber Entwicklungsgeschichte der Thiere. Königsberg, 1828.

²⁵ Keibel, F., Arch. f. Anat. u. Phys. Abth., 1889.

²⁶ His, W., Arch. f. Anat. u. Phys., Anat. Abth., 1892.

²⁷ Kupffer, C., Studien zur vergleichenden Entwicklungsgeschichte des Kopfes der Kranioten. Heft 1. München u. Leipzig, 1893.

²⁸ Herrick, C. L., Jour. Comp. Neur., III, 1893, p. XVI.

believe that at all stages of development, the hypophysis and its corresponding fold of the brain-wall represent the morphologic cephalic end of the body and of the brain. It logically follows that the cephalic end is the point at which the dorsimesal and ventrimesal lines meet. The ventrimesal line is present from the beginning as the middle line between the neural plates. The dorsimesal line is that in which the original margins of the neural plates unite in closing the brain tube.

To redetermine the exact location of this point of union of the two lines, young specimens of both immammalia and mammals, including man as far as material was available, have been examined step by step from the open neural plates to the closed neural tube and until adult landmarks become unmistakable. From these observations reported in 1903²⁹ and 1904,³⁰ it becomes certain to me that the original margin or dorsimeson extends as far as the hypophysial fold of the brain (Figs. 3, 4). This is irrespective of the exact place where the final closure of the neural tube takes place. In all the higher forms examined this final closure is between the eye-stalks, its adult representative being the pre-optic recess. In torpedo, as Kupffer found in sharks, this point of final closure lies between the olfactory lobes. But even here, in earlier stages the original margin of the neural plate extended as in mammals to the hypophysial region. The difficulties of determining the cephalic end of the tube in lamprey and other forms having an original solid neural plate are, that when the cavity does form, it has seemed somewhat uncertain as to the location of the front of the tube. From my observations it seems that even in the lamprey the front of the tube can be placed at the hypophysis. In amphibia the large size of cells and the consequent thickening produce an obscurity as to the exact formation, but here again, the weight of evidence seems to show that the original cleft between the two sides of the neural plate extends to the hypophysial region.

The human embryo here under special consideration is the most illuminating of any specimen examined for the purpose of determining the exact point at which final closure takes place, because that event is delayed until the surrounding parts are so well developed that identification is unmistakable. At the point recognized by Mall as the neuropore, lying between the eye-stalks, a connection of brain and skin tissues exists and extends through a number of sections (100μ or more). In parts the arrangement of cells indicates that the margins have only recently united (Fig. 16). Figs. 1-8 show the relations of the neuropore, and the extent

²⁹ Gage, Susanna Phelps, Science, N. S., XVII, 1903.

³⁰ Gage, Susanna Phelps, Amer. Jour. Anat., IV, 1904, No. 2, p. VIII.

of the epidermic thickening. The thickened epidermis, separated from the brain, extends cephalad from the point of contact toward the olfactory region and hence away from the hypophysis (Fig. 7). Following still farther away from the hypophysis the olfactory region is found (Figs. 3, 4, 5), separated by a sharp fold from the eye-stalk. From the above, the only logical conclusion seems to me that this specimen gives positive evidence that the olfactory region of the brain is not its morphologic cephalic part, but that the eyes are relatively to the original margin of the neural plate cephalad of the olfactory region, *i. e.*, nearer the hypophysis. This is in contradistinction to the arrangement that has always been accepted namely, that in the vertebrate brain the olfactory region is the most cephalic, forming the first of the series. Even His after his acceptance of the demonstration of Keibel and his own statements concerning the neuropore ignored the logical conclusion as to the order of the parts in the series.

Following von Baer, Reichert, and Götte, Studnička³¹ has finally demonstrated that the olfactory lobes and the cerebrum are essentially dorsal and paired outgrowths from the neural tube. The present investigation confirms this and further places the eye-stalk and the retina in a similar category as dorsal paired organs serially in front, *i. e.*, toward the hypophysis from, the olfactory lobe. The natural corollary follows that the optic chiasma crosses the original margin or dorsomesal line; that it is in serial order with the pre-commissure, forni-commissure, callosum, supra-commissure, and post-commissure, each binding together paired dorsal organs, the chiasma being as truly dorsal as the post-commissure.

Nor is the conclusion above reached based merely upon logic. The study of a model made of a mouse in which the neural plates are cleft to the hypophysis show that in tracing the series of folds, which have relation to the margin, there are on each side: 1st, Hypophysial rudiments both in the skin and the brain, consisting of folds which reach the margin; 2d, A fold which ends in the outgrowing eye, and extends to the margin, while along the outer surface of this fold the skin is in direct contact with the brain; 3d, A flap or margin, still undifferentiated, lying between the 2d fold and the mesencephal. By comparison of this specimen with later stages of various specimens, it is seen that the flap (3d) becomes differentiated into olfactory, cerebral, and diencephalic rudiments.

A model made of the neural plates of the human embryo 12 of the Johns Hopkins University collection, lends strong confirmatory evidence

³¹ Studnička, F. K., Kgl. böhm. Ges. Wiss., Math.-natur., XIV, 1901; Zool. Centralbl., VIII, 1901.

to the above observations, while the comparative studies on lower forms lend their quota to the result.

These conclusions were stated by me in the above-mentioned abstracts.²⁹⁻³⁰ Johnston,³² the latest reviewer of the serial order of segments of the head, does not agree with my view. His work is largely based on mammalian material which in my experience, as above stated, does not show the facts clearly being obscured by either thickened walls or secondary formation of a neural cavity. The crucial point on which he rests the conclusion that the olfactory is in front of the eye is dependent upon those observations which tend to show that; (1) The segmental mesoderm extends past the eye to the olfactory region; (2) The hypophysial thickening of the skin is continuous with the nasal epithelium in petromyzon. With regard to the first point attention is called to a recent paper by Froriep³³ in which he shows that the original mesoderm of the head does not primarily pass cephalad of the hypophysis upon the mesal line. As to the second point, Lubosch³⁴ shows that the thickened epithelium of hypophysis and olfactory plates is not continuous but is separated by an interval of thin epithelium. Moreover, it is shown incidentally in his figures, that it is in close connection with this thin mesal plate that the incipient cavity of the eyes originates, that is between hypophysis and olfactory.

In order to make his contention good, Johnston is driven to the conclusion that the eye is a dorsal organ lying between the olfactory region and the diencephal and in the course of development, is dragged ventrad to its final position, the cerebrum and the eye being portions of the same neuromere. The observation that the eye vesicle is originally at the edge of the neural plate between hypophysis and olfactory region seems to make this device unnecessary.

The question of the cephalic end of the brain is not, to what point the neural plate is cleft after the formation, and final growth of the mesoderm into the head, but to what point it was cleft at the outset. As above stated, in mammals and torpedo, the cleft originally extends to the hypophysis. The eye lies next to the hypophysis and distinctly intervenes between hypophysis and olfactory region.

More recent investigations on invertebrate brains,³⁵ seem to have established the fact that the lobe of the brain connected with the com-

²⁹ Johnston, J. B., *Jour. Comp. Neurol. & Psychology*, XV, 1905.

³⁰ Froriep, A., *Anat. Gesell., Verhandl.* 16, 1902.

³² Lubosch, W., *Morph. Jahrb.*, XXIX, 1902.

³³ Comstock, J. H. and Chujiro Kochi, *Am. Nat.*, XXXVI, 1902. They summarize the work, including Patten's, from 1775-1900.

pound eyes is really cephalad of that connected with the antennae, now proved to function as organs of smell. This fact seems to fit into the finding given above on the serial order of parts.

Total folds.—In Figs. 3, 4, it is seen that the neural tube is divided more or less clearly into lobules and these again into folds. Those now under consideration are not the total folds of the cerebrum considered by some authors as transitory fissures and by others as artifacts. Indeed, some of them antedate the distinct formation of a cerebrum, some being formed in early human specimens with open neural plates (No. 12 of Johns Hopkins University Collection, and No. 714 of the Harvard University Embryological Collection). In the present study, the cerebrum itself is the name applied to one of these total folds.

Nor are they newly recognized structures. Bischoff³⁸ in 1845 published a minute figure of the brain of an embryo dog showing such folds in the oblongata.

Total Folds in the Immammalia.—Orr,³⁷ in 1887, found in the lizard a series of such total folds. McClure³⁸ followed with similar results. Loey's³⁹ remarkable dissections of shark and also of chick and of *Amblystoma* show the beginning of these folds as marginal structures before the mesoderm had reached the parts, and therefore indicating the segmentation of the epidermis antecedent to that of the mesoderm. Loey's results have been questioned by Neal,⁴⁰ but in going over some of the same ground it seems to me that Loey's observations are well founded. The careful confirmatory work done in Loey's laboratory by Hill⁴¹ seem to put the essential points beyond controversy. He shows 3 neuromeres in the fore-brain, 2 in the mid-brain, and 6 in the hind-brain.

Orr, McClure, and Loey call the folds, neuromeres. This term is here avoided because it leads too far afield into a consideration of related questions of theory and fact concerning nerve distribution and mesodermic segments on which the literature is extensive and well-known.

Total Folds in Mammals.—In mammals, since the time of Bischoff,³⁸ figures of such folds appear occasionally in literature. Mihalkovics⁴² shows folds in the rabbit's oblongata; Prenant⁴³ in that of the pig.

³⁸ von Bischoff, T. L. W., *Entwicklungsgeschichte des Hunde-eies*. Braunschweig, 1845.

³⁷ Orr, Henry, *Jour. Morph.*, I, 1887.

³⁸ McClure, Chas. F., *Jour. Morph.*, IV, 1890.

³⁹ Loey, W. A., *Jour. Morph.*, XI, 1895.

⁴⁰ Neal, H. V., *Bull. Mus. Comp. Zool.*, Harvard Coll., XXXI, 1898.

⁴¹ Hill, C., *Zool. Jahrb.*, Abth. f. Anat. u. Ontog. d. Thiere, XIII, 1900.

⁴² Mihalkovics, V. von, *Entwicklungsgeschichte des Gehirns*. Leipzig, 1877.

⁴³ Prenant, A., *Soc. de la science de Nancy, Bull.*, Ser. 2, IX, 1889.

Zimmermann⁴⁴ in rabbit and chick shows 2 in the fore-brain, 3 in the mid-brain, 8 in the hind-brain, and 4 in connection with the accessorius nerve. Froriep⁴⁵ found in the mole 3 in the diencephal, 3 in the mid-brain, which disappear later, and 7 in the hind-brain, the last being connected with the vagus nerve. Schultze⁴⁶ figures folds in the pig. Lewis⁴⁷ and Minot⁴⁸ show four neuromeres in the oblongata of the pig. Bradley⁴⁹ is the latest investigator to study these folds in the pig, finding 7 in the hind-brain, the 1st belonging to the cerebellum and the 7th connected with the Xth nerve.

Total Folds in Homo.—Kupffer⁵⁰ merely mentions in a human embryo of three weeks, five pairs of total folds in the oblongata. His⁵¹ shows that in the region of the oblongata, certain folds in disappearing, leave behind cell-nests as in the olive. However, in his monumental work on human embryology,⁵² he seems to have avoided giving any hint that such structures exist, while in his models of early specimens, a glittering smoothness occurs in regions which are really full of significant form. In models of human embryos made in Dr. Mall's laboratory, certain facts shown in his specimens were avoided since they did not bear on the subjects he was investigating. However, this seems to be a case where a little positive evidence more than counterbalances a vast amount of silence.

Granting the existence of total folds in the neural tube of mammals at certain stages of development, the question has been put, are they artifacts due to shrinkage of the mesoderm? In answer I would say:—
1st. The crowded cellular growth of the neural tissue and the scattered cells of the mesoderm would seem to indicate that though the latter might shrink away from the neural tube, it would not throw it into such sharp foldings as occur. Direct observation seems to corroborate this argument.
2d. The folds are found at the margins of the neural plate in man before the mesoderm has grown up to this margin. 3d. In lower mammals corresponding in general to this human specimen, the mesoderm as far as

⁴⁴ Zimmermann, W., Anat. Gesell., Verhandl. 5, 1891.

⁴⁵ Froriep, A., Anat. Gesell., Verhandl. 6, 1892.

⁴⁶ Schultze, Oscar, Grundriss der Entwicklungsgeschichte des Menschen u. der Säugethiere. Leipzig, 1897.

⁴⁷ Lewis, F., Amer. Jour. Anat., II, 1903.

⁴⁸ Minot, C. S., Laboratory text-book of Embryology. Phila., 1903.

⁴⁹ Bradley, O. C., Rev. Neurol. and Psychiatry, II, 1904.

⁵⁰ Kupffer, C., König. baierische Akad. der Wiss., Math.-Phys. Cl., Sitz. XV, 1885.

⁵¹ His, W., König. sächs. Ges. d. Wiss., Abhand. d. Math.-Phys. Klasse, XVII, 1890.

it has grown around the sides of the neural tube shows little indication of shrinkage and forms complete contact with the foldings of the tube, indicating that the folds give form to the mesoderm rather than the reverse. 4th. Many of the young mammalian specimens examined are cut so accurately, after such perfect preservation, that no question of asymmetry can be raised as might be the case with the human material examined. It may be said that in all the models made by me, no matter how twisted or imperfect the specimen, the evidence of essential symmetry is clear. 5th. As is natural to suppose, the folds arise step by step with the growth and development of the tube. In human specimens they are in their most typical condition during the third week, after which their external creases are bridged by the growth of white matter and later their internal sharp lines are gradually obliterated. To realize the existence and probable significance one should study them when most typical and when they all have approximately the same size as in this specimen (148).

Serial arrangement of folds.—It must not be understood that the table given below represents a final conclusion as to the number of folds in the brain tube. It is an attempt to bring into as nearly definite relations as possible at this time, the early structures shown, with those of the adult. The lobules and folds cannot be said to accurately represent the definitive segments which Wilder³² proposes for convenience in studying later stages, nor do they more nearly coincide with the divisions settled upon by the German committee on Anatomical terms. In fact could we start without so many preconceptions from the complicated adult structure and the names which have been applied to the parts our task would be simpler. As it is, in the figures, as few names as possible have been used and even these do not always agree with the customary usages; for instance, the term Diencephal is here used for a part of the roof and lateral wall, but does not, as usually understood, include the infundibulum and the eye-stalk. Perhaps the old and indefinite term *thalamus* would better fit the case.

The common characteristic of all these folds (except the albicanthal, see below), is that each pair takes its origin in a common pocket at the dorsomeson (see above Cephalic End of Brain Tube), or at the edge of the membranous roof or metatela and thence radiates a greater or less distance along the lateral wall of the brain. Each fold, no matter how obliquely, tends in general direction across the axis of the brain tube.

³² Wilder, B. G., Reference Handbook of the Medical Sciences, 2d Ed., Vol. II, 1901. See also Bibliography in Wilder Quarter Century Book.

TABLE II.

Lobule.	Name given to fold.	Ref. No. in notes.	Abbreviations.	Figs. in which shown.	Associated structures.
I, Infundibular	{ Albicantial Hypophysial	a 1	alb., albi. infund.	3, 4, 6, 8, 9, 16 3, 4, 6, 8	Hyp.
II, Visual	{ Eye-stalk Eye-vesicle	2 3	eye	3, 4, 5, 9, 16
III, Cerebral	{ Striatal Olfactory Cerebral	4 5 6	str., stri. olf. cer.	3, 4, 5, 7
IV, Thalamic or Diencephalic	{ Diencephalic " 2 8	1 7	Dien.	3, 4, 5, 7, 8
V, Mesencephalic	{ Mesencephalic " 2 10	1 9	Mesen.	4, 5-10
VI, Cerebellar	{ Cerebellar " 2 12	1 11	cbl.	1
		2	"	2	
VII, Gasserian or Pontial	{ Oblongata " 3 15	1 13 2 14 3 15	obl. " "	1 2 3	3, 4, 10, 11
VIII, Otic	{ Oblongata " 5 17	4 16 5 17	obl. "	4 5	3, 4, 10
					{ VII, VIII Ear
IX, Vagal	{ Oblongata " 7 19	6 18 7 19	obl. "	6 7	3, 4, 11 3, 4, 13-15
X, Accessorial	{ Oblongata " 9 21	8 20 9 21	obl. "	8 9	3, 4, 13, 14
					{ XI N. XI N.

NOTES ON THE TOTAL FOLDS.

Albicantial.—In early stages of the chick and amblystoma, the first folds to form in the cephalic region before the neural plates close, is this pair lying at either side of a middle piece which is molded over the cephalic tip of the blind cephalic end of the enteron. This connection is soon lost, a great mass of mesoderm filling the cephalic bend, and intervening between the albicans and the pharynx (Fig. 3, 4). The prominence of these folds in this specimen (Figs. 9, 16) is especially marked. In mammals, they seem to decrease in relative prominence as the gill region begins to transform.

In many studies which have been made of the brain, a fold from the infundibular region is shown to extend entirely across the brain tube to

the roof of the diencephal. In this specimen, at first glance, it seemed that the only one from this region which could possibly extend to the roof of the diencephal, is the albicantial. The early history of the fold as seen above, does not make the interpretation seem probable and, moreover, a careful modeling of the region as shown in Figs. 6, 8, 9, seems to indicate that the albicantial and the diencephalic folds, originating at widely separated parts of the brain in the middle line, end near each other in the lateral wall but are distinctly not continuous.

This albicantial fold is only tentatively put at the beginning of the series, since from its original close approximation to entodermal rather than ectodermal tissue, it does not seem to belong to a truly dorsal series nor to be the ventral end of a diencephalic fold.

1. *Hypophysial*.—These folds are strongly developed in later human embryos. They are not sharply outlined in this specimen but the pair can be distinguished lying opposite to the pair of widely open pouches representing the hypophysis at this period (Fig. 9). There seems to be no fact thus far found which might bar these folds from the series. In a young mouse (see above), the folds as modeled show distinct relation to the margin of the neural plate, while the associated organ, the hypophysis, is a really paired organ⁵³ from the ectoderm as distinguished from the early entodermal relationship of the albicans. In this connection, the position of the hypophysial as the first in the series, it is significant that Boeke⁵⁴ finds in *Amphioxus* and certain fishes a ciliated pit in the region of the infundibular process, having according to him, the physical appearance of an organ of sense. Should there be confirmation of this it would represent a lost sense organ, the first of a dorsal series.

2. *Optic or Eye-stalk*.—In its earlier stages, this region is represented by a pair of wide furrows extending from the margin of the neural plate to the pouches forming the optic vesicles. With the closure of the plates, each furrow forms a wide vesicle connected in the present specimen, with the epidermis through the neuropore (Figs. 4, 6, 8, 16). As development proceeds, the portion of this vesicle toward the hypophysis, is constricted by a complicated folding to form the so-called optic nerves, the portion toward the olfactory remaining single as the pre-optic recess.

3. *Eye-vesicle or eye proper*.—As shown in Figs. 6, 8, and 9, the eye is distinctly constricted off from its stalk. In Figs. 6-8, it shows secondary folding, but a typical cupping does not occur.

The eye-vesicle seems to have relationship with the original margin,

⁵³ Gaupp, E., Arch. f. mikr. Anat., XLII, 1893.

⁵⁴ Boeke, J., Anat. Anz., XXI, 1902.

only through the stalk, the two together forming a lobule. Even at the point where the eyes approach the skin, they are separated from it by a thin layer of mesoderm (Fig. 16). The lens thickening has only a slight development, showing no tendency to bend towards the eye. Its borders are ill-defined.

4. *Striatal*.—A deep fold separates the visual region from the cerebral. On the side of the fold toward the cerebrum is a smaller total fold crossing the middle line (Fig. 4). From a careful comparative study this is identified with the fold later bordering the striatum.

5, 6. *Olfactory and Cerebral*.—In Figs. 4, 7, are shown the slight total folds, the forerunners of the olfactory and cerebral regions proper. Each pair of folds, begins in a mesal pocket but does not pass far across the brain tube. Mall⁵⁵ considers these among the artificial fissures of the cerebrum but in fact only one of them is cerebral and it represents the whole of that organ.

Relatively to the dorsimesal line, the three folds included in the cerebral lobule are seen to be caudad of the eye or as expressed by Studnička,⁵⁶ they are dorsal.

While the neural plate is still open it was found both in man and mouse that the region of the cerebrum and also of the diencephal (see above, Cephalic End of Brain Tube) is comprised in a narrow undifferentiated flap beyond the eye and including a portion of the margin. The flap becomes relatively wide before closure and shows some total folds which need more careful identification.

The olfactory epithelium shown in Fig. 3 has an irregular H-shape with a bar across the meson. This seems to agree with the idea that the olfactory epithelium shifts from the margin to its final, lateral position. Bedford⁵⁷ has in the pig, found a certain amount of lateral shifting of the olfactory plate. van Wijhe⁵⁸ found that in shark, both olfactory organs and nerve arise out of the neuropore, thus lending confirmation to the fact shown in this specimen.

7, 8. *Diencephalic*.—Are the two folds seen in the roof of the diencephal, each meeting its fellow of the opposite side, in the dorsimeson. 7, is in the region which ultimately forms the membranous roof and 8, is apparently to form the epiphysial outgrowth from its mesal pocket (cf. Minot⁵⁸).

⁵⁵ Mall, F. P., Amer. Jour. Anat., II, 1903.

⁵⁶ Bedford, E. A., Jour. Comp. Neur. & Psych., XIV, 1904.

⁵⁷ van Wijhe, J. W., Zool. Anz., IX, 1886.

⁵⁸ Minot, C. S., Science, N. S., XIV, 1901.

9, 10. *Mesencephalic*.—The roof of the mesencephal or mid-brain is in this specimen so broken that details of form were impossible to work out, but in general, it is possible to see that there are two pairs of total folds, one, the 9th, beginning in a mesal pocket lying caudad of the deep notch dividing the diencephal from the mesencephal, or in other words, caudad of the future post-commissure and extending obliquely caudad for half the length of the mesencephal, the other, the 10th, arising near the cephalic border of the metatela and ending abruptly near the point where the IVth nerve will take its origin (4 of Fig. 10).

12. *Cerebellar*.—These are two total folds represented clearly only in Fig. 11, rising at the cephalic border of the metatela and involving the part of the lateral wall which at this stage of development represents the cerebellum. Their history has not been traced.

14-18. *Oblongata*.—Are folds which arise at the edge of the membranous roof of metatela, and extend across the brain wall near to the ventrimeson. If one remembers that the neural plate on closing in this region as well as in the fore-brain, at first, is a tube with as thick walls dorsally as elsewhere, it is easily seen how the origin of these folds at the edge of the membrane, may represent the dorsomesal pockets occurring farther cephalad, especially since Locy's³⁹ work shows marginal folds in the early stages.

13. *Oblongata 1*.—In this and several other specimens studied, this fold seems fully separated from the following, obl. 2, but the sections are so cut as to make it difficult to trace it with certainty to the dorsal edge. In other human specimens studied I was not certain of its presence. In this region some authors find a ventral representative of the cerebellum, but in this specimen, at least, there is no such relation.

14. *Oblongata 2*.—This fold is one of the most strongly defined of the series. Its invariable connection with the roots of the Vth nerve and the Gasserian ganglion makes it a land-mark in the embryos of all vertebrates studied. The Gasserian ganglion is large but loosely formed and penetrated by branches of the jugular vein.

15. *Oblongata 3*.—This is also a sharply defined fold and has been widely recognized though as yet no structure has been definitely associated with it.

16. *Oblongata 4*.—The roots of the VIIth and VIIIth nerve are as invariably associated with this fold as the Vth with its fold. In the present specimen, in its dorsal portion, it is divided into two folds, the more cephalic being connected with the roots of the VIIth, the more caudal with those of the VIIIth nerve. The roots are very short, soon uniting with their corresponding ganglia. The ganglia lie close together

yet are for the most part distinguishable, the auditory following the thickening of the auditory vesicle on the cephalic and lateral portions, that of the VIIth extending without special differentiation into its nerve which forms a union with thickened epithelium at the dorsal end of the 1st gill-cleft (Figs. 1, 11).

17. *Oblongata 5.*—In the chick and all mammals examined, this fold lies opposite the otic vesicle but has no connection with it, unless possibly at a very early stage while the ear is merely a thickened plate pushed close to the neural plate. Dr. Johnston called my attention to the fact that such a fold is wanting in *Amblystoma* and an examination of material at hand, confirms his observation that no fold exists between that to which the VIIIth and IXth nerves are attached, in early stages of *Amblystoma*. However, in the shark Sewertzoff⁵⁹ shows such a fold to exist and it seems probable that modelling of the region in *Amblystoma* might reveal its rudiment.

18. *Oblongata 6.*—In all forms examined, this permanent fold has been found connected with the roots of the IXth nerve.

19. *Oblongata 7.*—This is a large fold arising at the caudal end of the metatela, extending obliquely cephalad and ending in the floor of the oblongata in close relation to the previous fold. It is connected with the roots of the Xth nerve. Apparently in other specimens, this fold cannot be so clearly defined as the others in the oblongata since it has rarely been recognized. Froriep⁴⁵ in one human specimen, and Bradley⁴⁹ in the pig, have observed this fold.

20, 21. *Oblongata 8, 9.*—These two pairs of folds are really dorsal pockets extending only through the dorsal half of the neural tube. Opposite their ventral portion, roots of the XIth nerve arise (Fig. 14). This nerve is interrupted in its course to join the Xth nerve by masses of ganglionic cells.

The relations of the VIIth, IXth, Xth, and XIth nerve of this specimen to their ganglia and the sensory epithelium have so recently been fully discussed by Streeter⁶⁰ that they will not be treated. The sensory epithelial thickenings to which he calls attention, are here figured (Fig. 1).

Beyond the clearly formed folds, above discussed, there occur several others each corresponding with an enlarged part of the ganglionic cord. As this cord has no further indication of dorsal nerve roots, the exact relations cannot be determined. Moreover, the following total folds in

⁵⁹ Sewertzoff, A. N., Anat. Anz., XXI, 1902.

⁶⁰ Streeter, G. L., Amer. Jour. Anat., IV, 1904.

the myel are not strongly marked, and in other specimens it is only in favorable sections that they can be seen at all.

THE SPECIAL POINTS APPEARING FROM A STUDY OF THIS EMBRYO ARE:—1. Both external form and internal organs show with diagrammatic clearness a normal development but with individual differences from other specimens of about the same age, some of these differences indicating greater, some less development. It seems probable that a careful study of such embryonic peculiarities in man and higher mammals may throw light on very important questions of heredity and variation.

2. Epithelial thickenings occur at the neuropore, olfactory region, lens, gill-clefts, and about the mouth, at the summit of the limbs, the thickening of the leg being continuous with that of the anal region.

3. There are 29 myotomes, 2 being occipital, and also remnants of 3 other occipital myotomes.

4. The nephric system is in a generalized condition presenting a recapitulation in one specimen of several distinct stages of development. This is shown by:—An open pronephric tubule on each side, independent of the Wolffian duct; each mesonephros having in its cephalic half, 8 rudimentary glomeruli opening by tubules into the duct; in its caudal half, 11 or 12 tubules not opening into the duct, but part of them opening to the coelom. The mesonephric tubules vary in structure from solid masses of cells to tubules with glomerulus and Bowman's capsule.

5. The developmental stage of the central nervous system shows with definiteness the position of the neuropore and its relation to the hypophysial region. In comparison with other specimens examined this makes it possible to determine the front end of the brain tube and of the body.

6. I believe that the morphologic cephalic end of the body is as figured by von Baer, in the region of the hypophysis and, furthermore, I believe as a generalization, that in all stages of development the hypophysial region is at the morphologic, cephalic end of the body, and consequently that parts which in the exigencies of growth have gone beyond this point are morphologically caudad of it, as the eye and olfactory region.

7. The brain tube shows both at this stage and at earlier and later stages total foldings which are directly correlated with definite nerves or epithelial thickenings. Other foldings have not yet been correlated with definite organs. These foldings are so uniformly present in mammals, birds, and selachians that they cannot be conceived of as artifacts but are believed to be true morphologic features.

NAMES AND ABBREVIATIONS USED ON THE FIGURES.

alb. or *albi.*—Albicans, albicantial fold.

allant. st.—Allantoic stalk.

amn.—Amnion.

anal pl.—Anal plate.

ao. ar., *1st-4th.*—Aortic arches, 1st to 4th.

A. umb.—Arteria umbilicalis.

aur.—Auricle, left, right.

auric-vent. c.—Auriculo-ventricular canal.

A. vit.—Arteria vitellina.

b. art. or *b. arter.*—Bulbus arteriosus.

B. c.—Bowman's capsule.

bile d.—Bile duct.

cbl.—Cerebellum.

cbl. 1, 2.—Cerebellar fold, 1, 2.

cer.—Cerebrum.

1st-8th cer.—1st to 8th cervical myotomes.

ch.—Chorda.

d. Cuvier.—Duct of Cuvier.

Dien.—Diencephal.

duod.—Duodenum.

g. c. 1st-4th.—Ectodermal gill clefts.

glom.—Glomerulus.

gn.—Ganglion.

gn. Fror.—Froriep's ganglion.

gn. Gsn.—Gasserian ganglion.

g. p. 1st-4th.—Entodermal gill pouches.

Hypoph.—Hypophysis.

inf. or *infund.*—Infundibulum or infundibular fold.

intest.—Intestine.

lach.—Lachrymal furrow.

lens ep.—Lens epithelium.

l. perit. cav.—Lesser peritoneal cavity.

mand.—Mandible.

max.—Maxilla.

Mesen.—Mesencephal.

mesent.—Mesentery.

mesoneph.—Mesonephros.

metat.—Metatela.

my. 1-32.—Myotomes.

N. III-XII.—Nerves III-XII.

nas. ep.—Nasal epithelium.

neur. or *neurop.*—Neuropore.

neph. or *neph. t.*—Nephric tubule.

obl. 1-9.—Oblongata folds, 1 to 9.

œs.—Esophagus.

olf.—Olfactory epithelium or olfactory fold.

pron. t.—Pronephric tubule.

s. venosus.—Sinus venosus.

sec.—Section.

sept. trans.—Septum transversum.

stom.—Stomach.

str., stri.—Striatum.

supra-ren.—Supra-renal capsule, adrenal.

t. 1-21.—Mesonephric tubules, 1-21.

V. jug.—Vena jugularis.

V. postcard. or *V. pc.*—Vena cardinalis.

V. umb.—Vena umbilicalis.

V. vit.—Vena vitellina.

vent.—Ventricle.

vit. ves.—Vitelline vesicle.

W. d., Wolff. d., Wolffian d.—Wolffian duct.

EXPLANATION OF PLATES.

FIGURES 1-13.

Drawings made from a model of Embryo 148 of the Mall collection, with sections and dissections of the same (see above, Models and Drawings). Magnification of the figures, $\times 33\frac{1}{3}$.

PLATE I.

FIGS. 1 AND 1A. View of the left side of the model. Compare with figures of this embryo in articles by Mall 1, 5.

It shows: The head comparatively small in diameter but great in length, and forming at the neck-bend an angle of 65° with the body; the position of the neuropore; the eye and ear scarcely apparent as external features; the prominent heart, limb buds, and tail; the umbilicus turning to the right (cf. Fig. 5); the wide undeveloped mouth and small maxillary process; the crowding of the 2d, 3d, and 4th clefts into the precervical sinus; and 29 myotomes, the 3d being noted as the 1st cervical.

The density of the stippling on Fig. 1 indicates the relative thickness of the epithelium (see above, External Form).

The topographic lines show the direction of the sections, the numbers

upon them indicate the corresponding sections of the series. The following figures have either topographic lines or the section number at which they are cut, and hence can be located with reference to Fig. 1.

PLATE II.

FIG. 2. A face view of the head. As shown by the topographic lines, it is tilted to give a clear view of the parts about the mouth which is merely a wide slit between the hypophysial region of the head (cf. Figs. 3, 4) and the mandibular process.

There are seen: The small maxillary process with the depression at the corner of the mouth lying between maxilla and mandible; the H-shape of the nasal epithelium extending also over the cerebral region; the large neuroporic thickening; the lens epithelium with a tract extending along the lachrymal furrow.

The cut surface (Sec. 125) shows: The division in the dorsal part of the auricles (cf. Fig. 3); the entrance of the sinus venosus into the right part (left of Fig.); the liver lying in the septum transversum; the folds about the duct of Cuvier, pushing across the space to help form the diaphragm; the connection of pericardial and abdominal cœlom; the opening of the lesser peritoneal cavity into the abdominal cœlom.

FIG. 3. A view from the left side showing the central nervous system, pharynx, heart, lung, and liver. The lateral wall has been removed. Projections of the ear vesicle and myotomes 1-15 are indicated by dotted outlines.

The approximately uniform tube formed by the central nervous system and the strong cephalic flexure characteristic of this stage of development are evident. The mesoderm in the flexure has been removed.

The brain shows: The series of total folds; the great prominence of the albicanial; the relation of the neuropore to the epidermis and to the visual lobe or eye; the small size of the striatal, olfactory, and cerebral folds; the relation of the fold, oblongata 2, to the Vth nerve root (shown by a dotted circle); of the fold, obl. 4, to the VIIth and VIIIth nerves (dotted circles); of fold, obl. 6 to N. IX; the great size of fold, obl. 7 and its relation to N. X; the continuity and segmented character of the ganglionic chain in the neck region with the roots of the XIth nerve extending along its dorsal side; and the folds in the myel.

There are seen: The inner tube of the bulbus arteriosus as it enters the floor of the pharynx and divides into the aortic arches; the median thyroid cephalad of this branching; lateral folds just cephalad of the thyroid, the only rudiments of the tongue present; the bursa pharyngea, the dorsal pocket at the division of trachea and esophagus (at left of abbreviation *ch.*); dotted lines indicating the outline of the epithelial tubes forming lung and alimentary canal; the wide communication of the pericardial and abdominal cœlom dorsad of the septum transversum; the point of union of the aortæ (aorta); the hypophysis cut to the left of the middle line.

FIG. 4. A mesal view of the brain, myel, and pharynx. The brain shows from the interior the same total folds as Fig. 3, but brings out somewhat more clearly the grouping of folds into lobules (cf. Table II, in the text). The elevations in Fig. 3 correspond to the depressions in Fig. 4.

Especially noticeable in this figure are: The short striatal folds; the apparent continuity of the albicantial fold with the roof fold (cf. Figs. 8, 9); the cerebellar folds 1 and 2 (cf. Fig. 11, *cbl.*); the cleft in the oblongata fold 4, on either side of which arise the roots of the VIIth and VIIIth nerves (shown by dotted circles); the cut ends of the ring of mesoderm surrounding the neuropore; the intrusion of mesoderm into the cephalic flexure; the fact that no mesoderm intervenes between hypophysis and infundibulum on the middle line; the union of all layers in the roof of the oblongata; the dark areas in the pharynx, indicating with their dotted extension the membranous parts of the 1st to the 4th gill pouches; the notochord touching the caudal wall of the hypophysis and coming in close contact with the roof of the pharynx between the level of the 1st and 3d gill pouches.

PLATE III.

FIG. 5. A ventral view of a segment of the model (Fig. 1) extending from section 247 to 155.

The left side of the head is dissected away to show: The relation of the visual lobe, the neuropore, the olfactory and cerebral regions, and the roof of the diencephal.

The right side of the head shows: The thickened epithelium of the neuropore; the olfactory region and its extension over the cerebrum; the future lens; the mandible and gill-cleft-like pocket at the corner of the mouth.

The caudal portion of the figure (Sec. 247) shows the 23d myotome apparently continuous with the mesoderm of the leg-bud; the division of the aorta into the umbilical branches, the left one looping over the caudal end of the coelom; the left umbilical vein with a branch from the leg; the right plexiform umbilical vein passing along the body-wall toward the heart.

The middle part shows: The wide umbilicus turning to the right and containing the thick-walled vitelline sac with its veins and arteries and its union with the caudal intestine inclosed in mesentery; the reappearance of the intestine in section near the union of the Wolffian duct and allantoic stalk (cf. Fig. 17).

FIG. 6. A ventral view of a deeper segment of the model. It extends from section 185 to 155. In the head region (cf. Figs. 2-5) it is cut through; the neuropore; the eye vesicles, partially constricted off from the stalk; the albicantial folds (cf. Fig. 9); and the cephalic end of the thick-walled mesencephal as it dips into the albicantial region. At N. III is a strand of mesodermic tissue but apparently no true nerve fibers.

The caudal part of the figure (Sec. 185) shows: On one side, the appearance of a myotome cut through the middle; on the other side the overlapping ends of two myotomes; the 8th mesonephric tubule opening into the Wolffian duct and with its cap of thickened peritoneal epithelium (cf. Fig. 17) and its artery and vein.

In the middle portion are: The umbilical veins at either side, the left showing the greatly divided sinuses; the vitelline veins as they approach on either side of the alimentary canal; the vitelline artery; the liver near its caudal part embedded in the transverse septum and near the level where the bile duct unites with the duodenum; the umbilicus turning to the right of the specimen.

The heart (Sec. 168) cut near its middle shows: The undivided chamber of the ventricle; a strong fold arising between the two sides and separating the exit of the bulbus arteriosus (cf. Fig. 12) from the entrance of the auriculo-ventricular canal (cf. Fig. 10).

FIG. 7. From a segment of the model cut at section 200, near the edge of the neuropore, looking into the roof of the curved brain tube and showing that the striatal, olfactory, and cerebral folds, and those of the roof of the diencephal and of the mesencephal, meet in the mid-dorsal line, there being no division by a middle partition.

FIG. 8. A segment of the model extending from Sec. 198 to 155. It cuts the neuropore (cf. Fig. 16), showing a pit on its neural aspect, and looks into the visual lobe and eye vesicles in the opposite direction from Fig. 7. It shows the notch at the tip of the optic vesicle, apparently the beginning of the optic cup.

The deep projection of the floor of the mesencephal into the albicanthal region is here shown and the independence of the albicanthal folds from those dorsad of it (cf. Fig. 9).

PLATE IV.

FIG. 9. A segment of the model from section 162 to section 200 (cf. Figs. 1-4), showing: A caudal view of the eye vesicle and visual lobe; the V-shaped union of the albicanthal folds and their independent dorsal ending; the mesencephal with its sharpened beak-like ventral ending between the albicanthal folds; the strand of tissue, at the point where the III N. would later appear; the hypophysis forming a bi-lobed, ectodermic organ surrounding the end of the hypophysial fold; part of the neuroporic thickening; and the Gas-serian ganglion.

FIG. 10. A segment of the model extending from Sec. 155, through the cephalic flexure, to Sec. 96. With dissections at Secs. 130 and 140. It shows: The base of the mesencephal and oblongata, with the large protuberance (4) at the end of the second total fold of the mesencephal; the oblongata folds 1-5, and the relations to the Vth and VIIth Ns.; mouth; pharynx; the ending of the gill-clefts 2-4 in the precervical sinus; the entrance of the vitelline veins into the liver at the side of the duodenum and their union in the dorsal part of the liver with the sinus venosus; the vitelline artery; and the mesonephros.

FIG. 11. A view from the dorsal side of the same segment of the model as is shown from the ventral side in Fig. 10, *i. e.*, it extends from Sec. 96 to Sec. 155 (cf. Figs. 1, 3, 4). It cuts the arm buds, looks into the floor of the pharynx and cephalad into the pons, mesencephal and ear vesicles.

There are seen: A portion of the cerebellum with its folds; the mesencephal with its narrow opening cephalad and its floor protruding deeply into the pons region; the interior view of the pons lobule with its three folds, obl. 1, obl. 2, obl. 3; the otic lobe showing the ventral ends of folds, obl. 4, 5; obl. 4 connected with the VIIth and VIIIth nerve; at the right the relation of the ear vesicle to obl. 5; at the left the ganglion of the VIIIth lying next the otic vesicle, that of the VIIth crossing dorsad of the first gill-pouch; at the right the intimate union with the epidermis of the ganglion of the IXth nerve; the ventral ends of folds obl. 6.

On the left, dissections down to Secs. 99, 103, 110, and 128 show: The relations of the gill arches, and the four gill pouches to the pharynx, the larynx, esophagus; the coelomic cavities separated by the mesentery and only partially divided into pericardial and abdominal regions by the lateral infolding formed by the ducts of Cuvier; at the left the dissected cardinal vein arching over the coelom and uniting with the jugular vein to form the duct of Cuvier and thence dipping ventrad to join the sinus venosus (cf. Figs. 10, 2); the right and left aortæ near their point of union; the right arm-bud with its thickened epithelium and the branches of the terminal blood-vessels; the 10th myotome merging into the mesoderm of the left arm-bud near its dorsal portion; well developed motor nerve roots.

FIG. 12. A ventral view of the heart showing the somewhat greater length of the right part of the common auricular chamber. The figure is labeled as though the right and left sides were separate. By comparison with Figs. 2-6, the relations are seen of the bulbus arteriosus and the sinus venosus to the single tube forming the heart.

PLATE V.

FIG. 13. A segment of the model (Fig. 1) from Secs. 5 to 20, showing: The total foldings obl. 7, 8, 9 of the neural tube dorsad of the roots of the Xth, XIth, and 1st cervical nerves (cf. Figs. 3-4); the foldings of the skin corresponding with those of the neural tube; the metatela rapidly widening from the cephalic end of the cut surface (cf. Fig. 14); the close connection of neural and epidermic epithelium.

FIG. 14. From a photograph ($\times 47\frac{1}{2}$) of Sec. 25 of the human embryo 148 (cf. Figs. 1, 2, 4). It shows: The neural tube just at the ventral border of the folds, obl. 8, 9, represented in Fig. 13; the cephalic, 1st, root of N. XI, attached to the base of fold, obl. 8; the 2d root of N. XI, attached to obl. 9; the XIth N. as it passes through the 1st cervical and Froriep's ganglia; the intimate union of the 2d and 3d cervical ganglia.

The cilia lining the tube appear faintly and stop short of the dorsal margin of the neural tube.

At the right, the first four myotomes are seen, the 4th showing especially well the dorsal division into two separate horns. Noticeable is a continuation cephalad of similar cell-groups and epidermal corrugations representing remnants of still more cephalic, occipital myotomes.

FIG. 15. A photograph ($\times 47\frac{1}{2}$) of part of Sec. 44 (cf. Figs. 3, 4), showing the neural tube with its cilia, metatela, and the relations of the Xth, XIth, and XIIth nerves.

At the left of Fig. 14, the fold, obl. 7, is seen. At the right, it has disappeared, to reappear as a more marked depression at the lower level of Fig. 15, where the attachment of the Xth N. is found.

At the left is seen the appearance of the 1st, 2d, and 3d myotomes at a lower level than in Fig. 14, and at the right, at a still lower level, as they recede farther from the skin and where the nuclei, related to the developing muscle fibers, form a band across the myotome. At the left is a continuation cephalad of the same segmented appearance of the epidermis as that which lies over the myotome.

FIG. 16. A photograph ($\times 47\frac{1}{2}$) of Sec. 192, through the neuropore to show: The point of most intimate union of the thickened epidermis with the neural epithelium (for the extent of the neuroporic thickening, (cf. Figs. 1-8); the total folds entering into the formation of the visual lobe; the sharp angle formed by the albicanial furrow at the right; the external filling up of this angle at the left, indicating its independent ending (see text); the mesoderm continuous over the eye-vesicle, but interrupted at the neuropore (cf. Fig. 4). The cilia present in this section do not show clearly here.

FIG. 17. Ventral view of a large model of the nephric region of Homo 148, extending from Sec. 94 to Sec. 295 ($\times 75$). It shows the two Wolffian ridges, each including the pronephric remnant; the mesonephros; the dorsal portion of the mesentery; a part of the stomach and lesser peritoneal cavity; the union of the allantoic stalk with the intestine; the cloaca and the imperforate anal plate; the right Wolffian duct and its union with the cloaca.

The opening of the pronephric tubules (Fig. 18) on the coelomic surface is shown. Crosses indicate the position of the first eight mesonephric tubules which do not open on the coelomic surface (Fig. 19) but connect with the Wolffian duct. The 9th to the 20th tubules have no connection with the Wolffian duct. The 9th and the 13th to the 18th are open to the coelom. The 10th to the 12th are hollow but connect neither with duct nor coelom. The 19th to the 21st are thickenings touching the coelomic epithelium but showing no cavity. The general arrangement of the tubules of the other side is similar but not identical.

The slight furrows at the cephalic end may represent a rudiment of the supra-renal or adrenal body.

FIG. 18. From a photograph ($\times 160$) of Sec. 104, Homo 148, showing the pronephric tubule of the right side (Fig. 17) with its opening into the coelom and its duct traceable for a few sections farther cephalad. The cardinal vein is also seen.

FIG. 19. From a photograph ($\times 160$) of Sec. 166 through the 7th mesonephric tubule of the right side (Fig. 17), showing the small rudiment of the glomerulus; the typical S-shaped tubule connecting with the Wolffian duct and thin-walled Bowman's capsule which a few sections farther caudad unites with the coelomic epithelium and forms a small opening. The remainder of the first eight tubules are of this same general type but are completely separated from the coelomic epithelium.

FIG. 20. From a photograph ($\times 160$) of Sec. 195, through the 10th mesonephric tubule of the left side (Fig. 17), showing a wide opening to the coelom and its independence of the Wolffian duct.

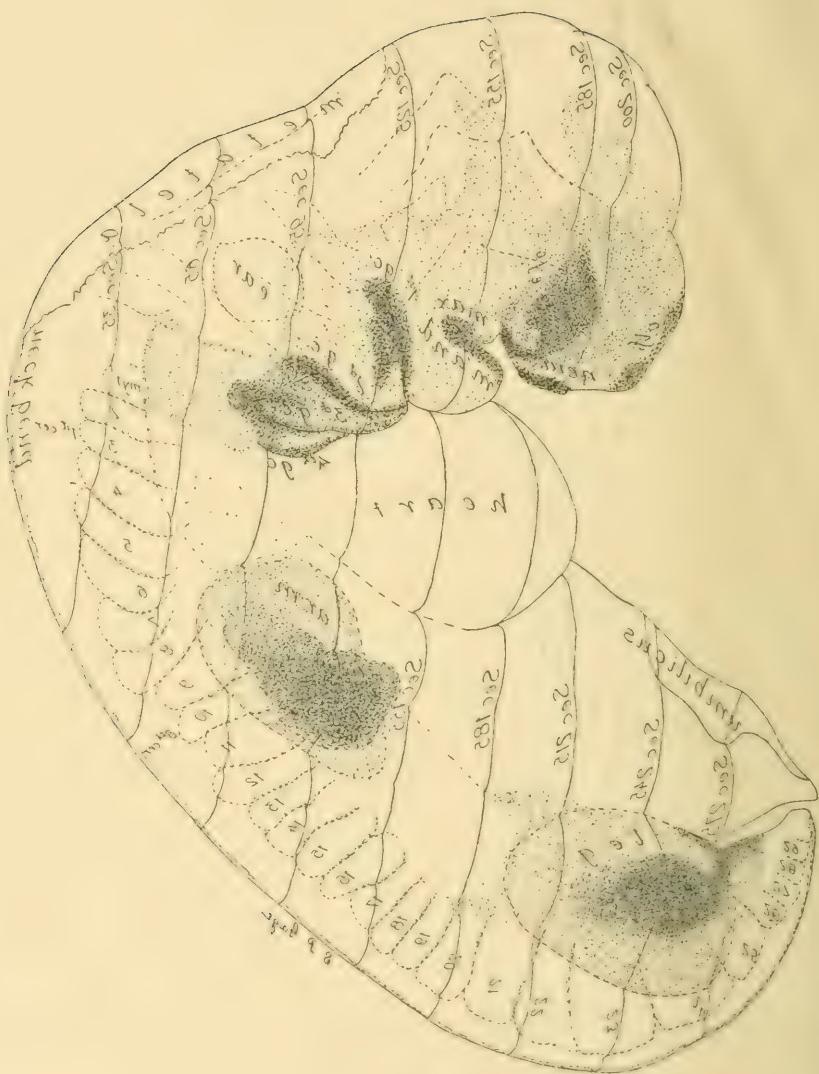
The photographs reproduced on this plate were made by Henry Phelps Gage and are part of a complete series of 96 taken from the sections.

A THREE WEEKS' HUMAN EMBRYO

SUSANNA PHELPS GAGE



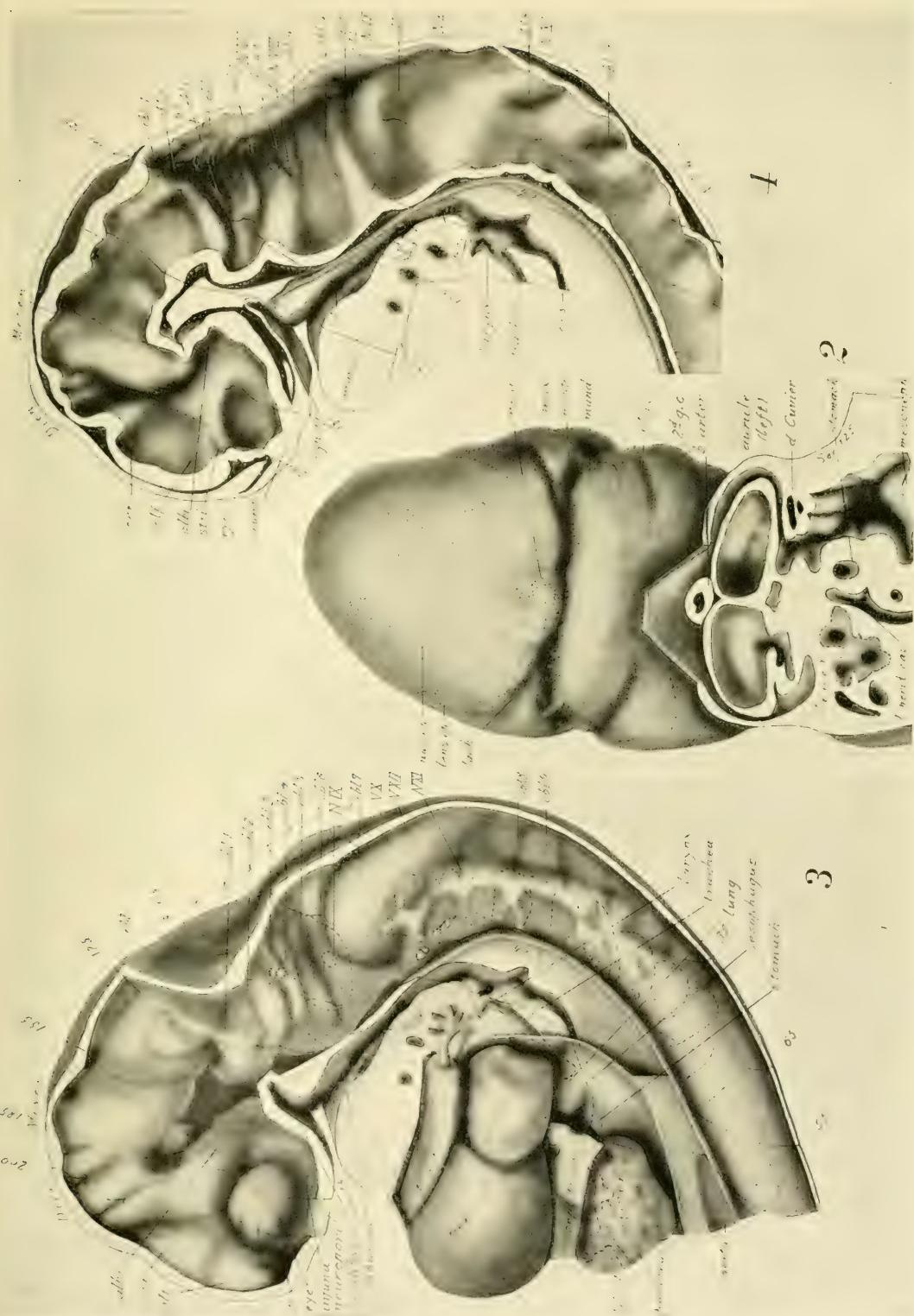
FIG. 1

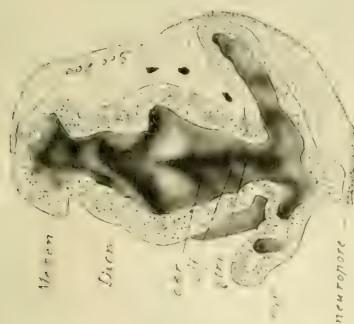


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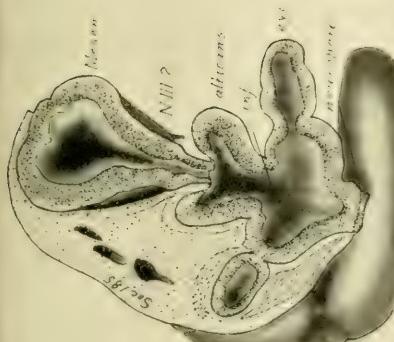




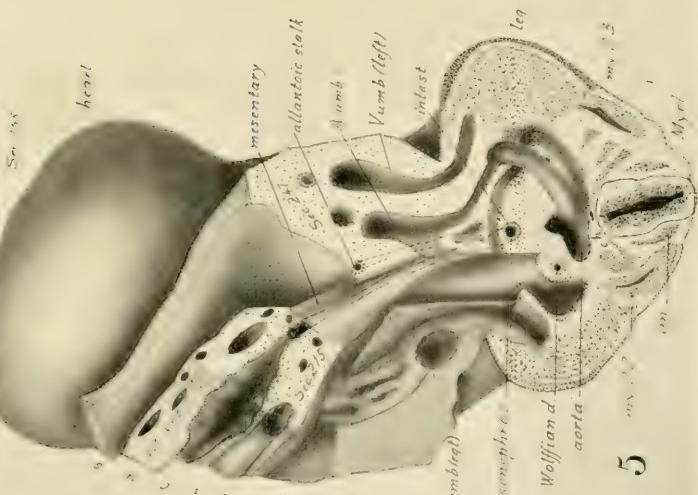
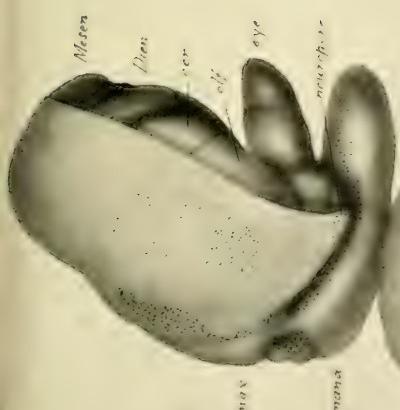
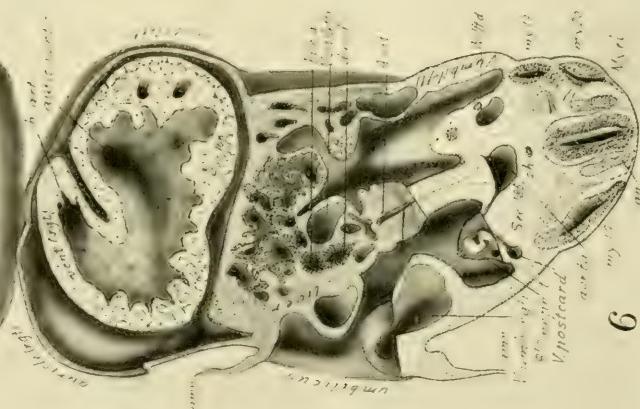
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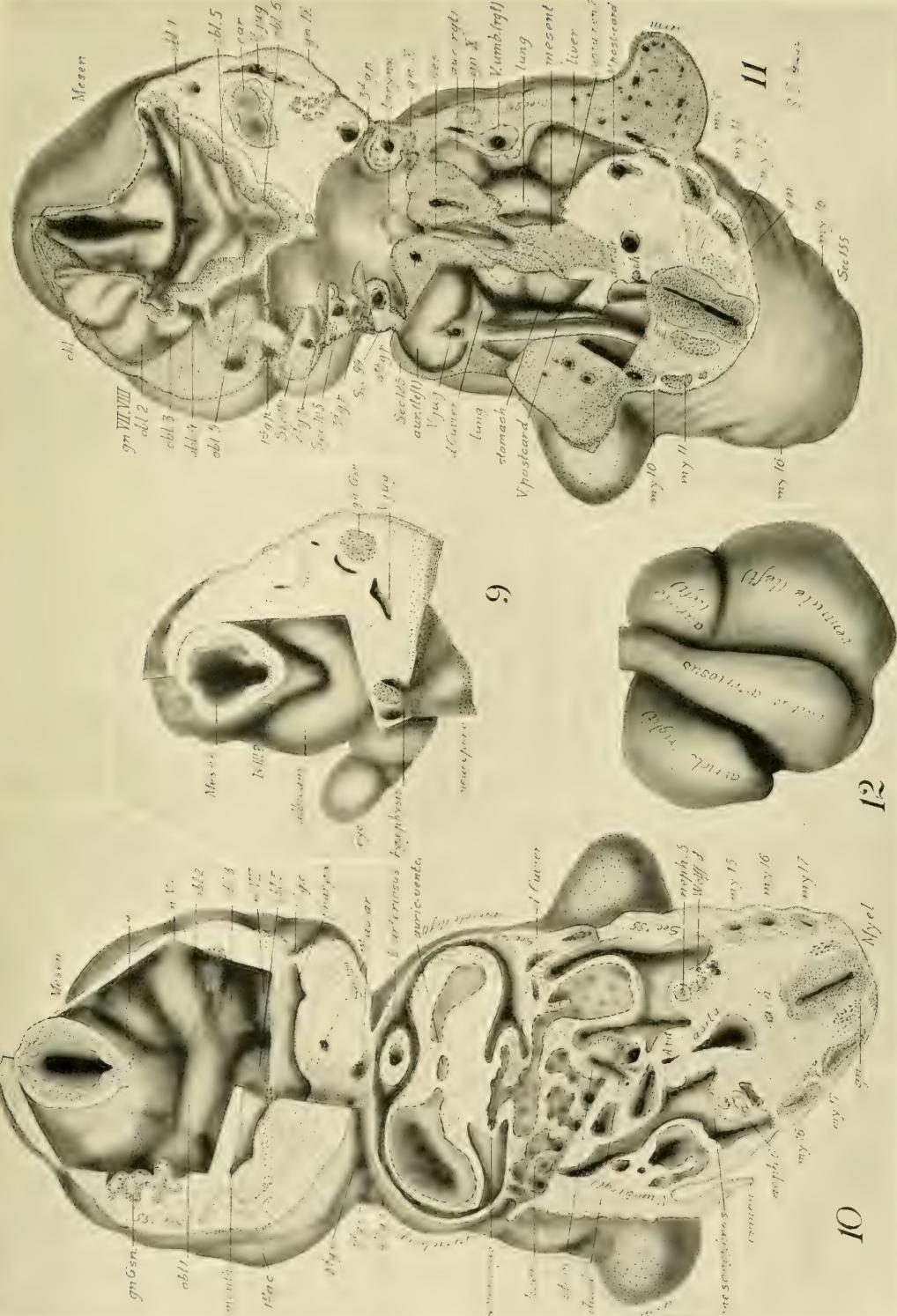


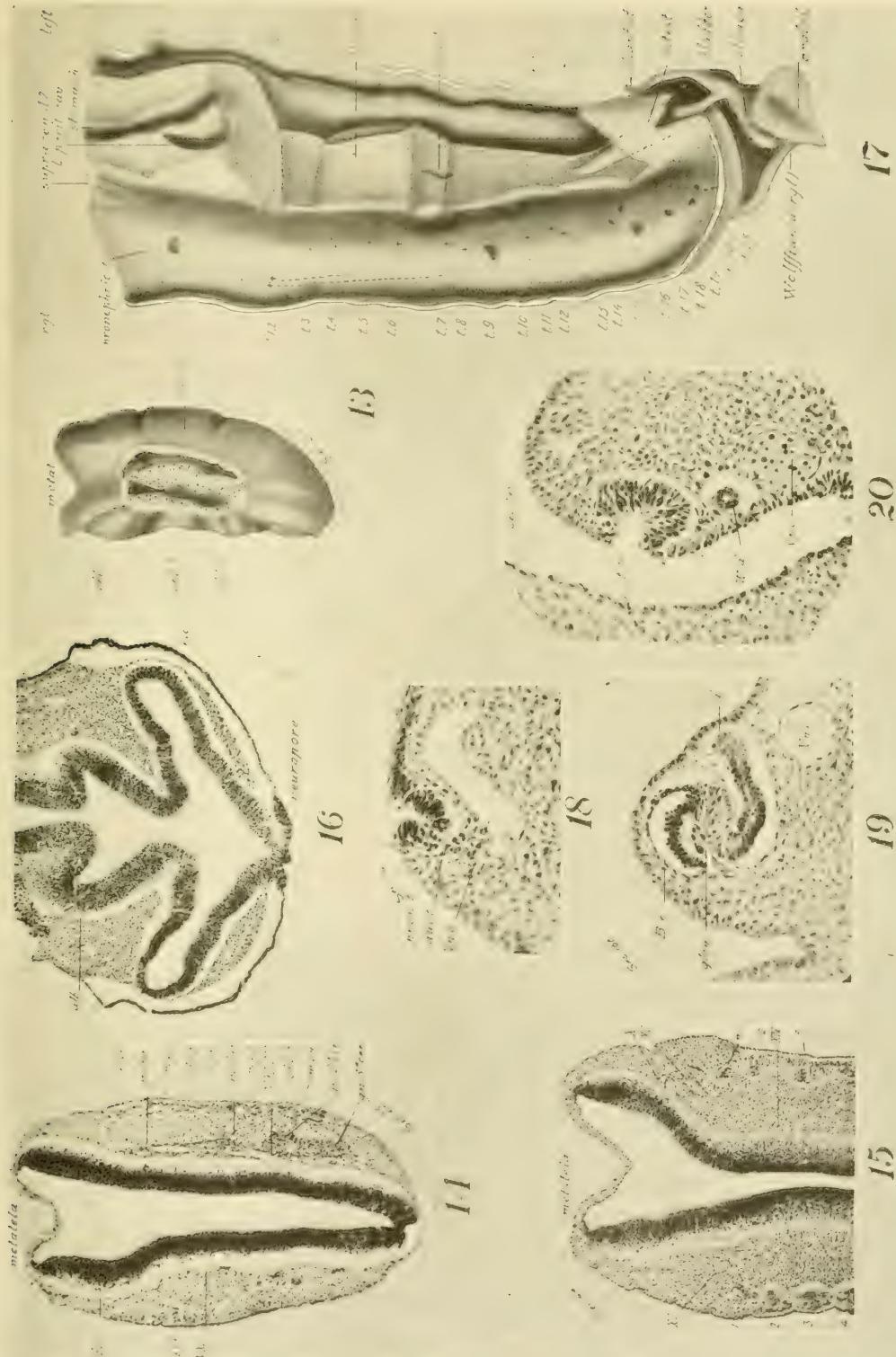
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SUSANNA PHELPS GAGE





THE BLOOD AND LYMPH VESSELS OF THE LUNG OF NECTURUS MACULATUS.

BY

WILLIAM SNOW MILLER.

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WITH 3 TEXT FIGURES AND 2 PLATES.

ANATOMICAL RELATIONS OF THE LUNGS.—The lungs of *Necturus maculatus* consist of two elongated cylindrical sacs which are situated one on each side of the body cavity. Anteriorly they meet in the mid-line and communicate by means of a narrow slit, the glottis, with the short, wide pharynx. No septa are present in the lungs and both inner and outer surfaces are smooth except for such irregularities as are occasioned by the blood vessels.

When fully distended the lungs measure, in an adult animal, from 80 to 100 mm. in length and from 7.5 to 10 mm. in diameter; they are slightly crescentic in shape (Fig. 1) and enclose an elongated oval space which is occupied by the posterior portion of the oesophagus, the spindle-shaped stomach and the anterior portion of the intestine.

Each lung is attached throughout nearly its entire length to a fold of the peritoneum; the right lung to one which extends from the liver to the mid-dorsal body wall, the left, to one which extends from the stomach to the mid-dorsal body wall. In an average sized animal only 15 or 25 mm. of the normally distended lung is free from this peritoneal attachment.

THE BLOOD VESSELS.—Three afferent branchial arteries convey the blood on each side from the heart to the gills. Each artery runs along the ventral border of the corresponding gill giving rise to numerous fine branches which break up into a capillary network. From this capillary network numerous radicles unite to form on the dorsal border of each gill an efferent branchial artery. There are thus formed on each side three efferent branchial arteries (Fig. 1, *E. B.*) which form by their union the right and left aortic roots (Fig. 1, *R. A.* and *R. A.'*) these, in turn, unite in the mid-line dorsal to the anterior end of the stomach to form the aorta (Fig. 1, *A.*).

The pulmonary artery (Fig. 1, *P. A.*) arises from the third (fourth) efferent branchial artery after it has been joined by the second efferent artery and lateral to the entrance of the anastomosing branch from the first efferent artery. It runs obliquely towards the lung, giving off along

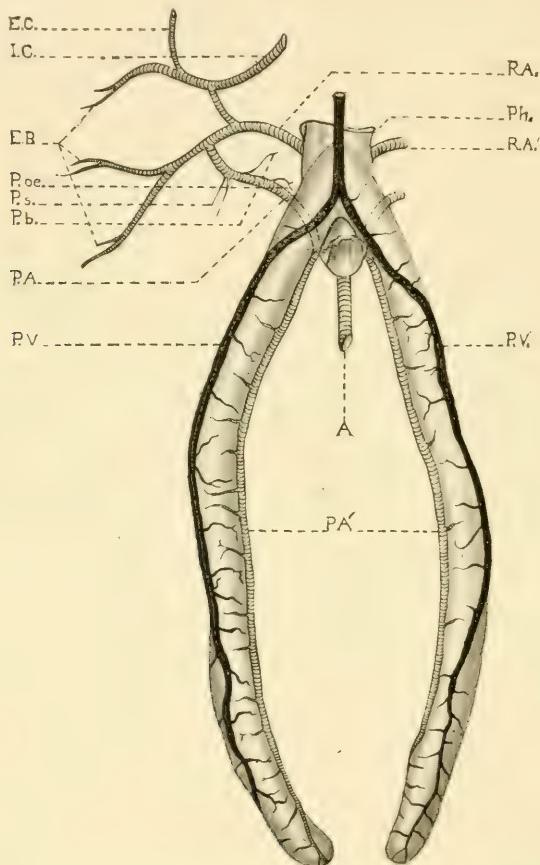


FIG. 1. The blood-vessels of the lung of *Necturus*. *A.*, aorta; *E. B.*, efferent branchial arteries; *E. C.*, external carotid; *I. C.*, internal carotid; *Ph.*, oesophagus; *P. A.*, *P. A'*, pulmonary artery; *P. b.*, *P. oc.*, *P. s.*, branches of the pulmonary artery outside of the lungs; *P. V.*, pulmonary vein; *R. A.*, *R. A'*, aortic roots.

its course small branches to the muscles of the shoulder, to the group of muscles lying ventral to the buccal cavity and to the oesophagus (Fig 1, *P. s.*, *P. b.*, *P. a.*). The artery now passes across the dorsal surface of the lung to gain its dorso-mesial side, along which it runs, gradually diminishing in size, to its tip (Fig. 1, *P. A.'*). Throughout the course

of the pulmonary artery in the lung lateral branches are given off, sometimes from opposite sides of the main trunk, more frequently, alternately from one side, then, the other. These branches are so arranged that, as a rule, an arterial radicle lies between two venous radicles (Fig. 1).

Arising from the main arterial trunk, and also from its branches, is a rich network of capillaries which is spread out over the entire inner surface of the lung; the venous radicles take their origin from this network (Pl. II). Not infrequently small branches of the main artery extend across one or the other side of the lung and enter directly into the main venous trunk. This direct union of artery and vein is quite common in some lungs, while in others it is entirely absent. In some instances two small arterial radicles will join the end of a venous radicle giving the appearance of a vein arising from the main artery by a forked extremity. Examples of each type can be seen in Plate II.

The pulmonary vein (Fig. 1, *P. V.*) extends along the ventro-lateral side of the lung. It is formed not only by the capillaries, into which the pulmonary artery breaks up, entering it directly, but also by venous radicles which take their origin from the same capillary network. As the lungs converge to unite on the ventral side of the oesophagus the pulmonary veins leave the side of the lung and cross it obliquely to meet and fuse in the mid-line into a single vessel (Fig. 1, *P. V.*, and Pl. I., Fig. 2). From this point of union the now single pulmonary vein runs anteriorly towards the heart; passing to the dorsal side of the two arms of the hepatic sinus it usually continues along the wall of the left arm of the sinus and opens into the left side of the atrium of the heart (Fig. 3).

This description of the pulmonary blood vessels differs very materially from that given by Suchard for Triton and Salamandra. Suchard reverses the course and position of the artery and vein from what I have found to be the case in *Necturus*; the artery, according to his description, occupying the position of the vein and the vein that of the artery.

In still another important particular does the distribution of the blood vessels in *Necturus* differ from that given by Suchard for Triton and Salamandra. In *Necturus* there is no interruption of the capillary network over the pulmonary vein; Suchard describes such an interruption in Triton and Salamandra. In *Necturus* the capillary network is spread out over both artery and vein.

THE LYMPH VESSELS.—Broadly stated, the lymphatics follow the blood vessels, both arteries and veins; their arrangement, however, about these vessels is different.

Along the course of the main artery three lymph trunks are usually found placed nearly equidistant from one another and connected together by numerous anastomosing branches (Pl. II). The main lymph trunks show great irregularity in size; sometimes, by their wide dilatations coming nearly into contact, the artery is practically hidden from view (Pl. I, Fig. 1). As the artery approaches the tip of the lung the number of lymph trunks are, in most lungs, reduced to two, placed one on either side of the artery. Numerous anastomosing branches connect the two lymph vessels.

Each lateral branch of the pulmonary artery as it leaves the main trunk is accompanied by two lymph vessels which arise from the main trunks, and like the main trunks they are connected together by fine anastomosing branches (Pl. II). The lymph vessels accompanying the branches of the pulmonary artery can be traced across the interval between the pulmonary artery and pulmonary vein to their union with one of the main lymph trunks about the pulmonary vein (Pl. II). When a branch of the pulmonary artery forms a direct anastomosis with the pulmonary veins there can usually be recognized two accompanying lymph vessels which join directly one of the main lymph trunks about the pulmonary vein.

At the root of the lung the network of lymph vessels about the pulmonary artery usually forms two large trunks which join the exceedingly rich network of lymph vessels in the wall of the stomach on its dorsal side (Pl. I, Fig. 1). In some animals small lymph vessels pass from the peri-arterial network about the left pulmonary artery along the dorsal mesogastrium to join this same network.

In some lungs one of the two main trunks above mentioned passes around to the ventral side of the lung and forms an anastomosis with the lymph vessels about the pulmonary vein (Pl. I, Fig. 1).

Hoffmann says in regard to the lymph vessels of the lung of *Rana*: "Die Lymphgefässe begleiten in der Lunge ausschliesslich die arteriellen, nie die venösen Blutgefässe." Suchard, in his description of the lymph vessels of the lung of *Triton*, says: "les branches du réseau péri-arteriel sont moins nombreuses et moins volumineuses que celles du réseau péri-veineux." We shall see that neither of these statements holds true for the lung of *Necturus*.

The main trunk of the pulmonary vein is accompanied by two large lymph trunks which are connected together by less numerous anastomosing branches than is the case with the lymph trunks about the artery (Pl. II). In some lungs they are apparently absent or for some reason they fail

to inject (Pl. I, Fig. 2). Each lateral branch of the pulmonary vein is accompanied by one or more lymph vessels, but the arrangement is not as regular as that about the branches of the pulmonary artery (Pl. II).

The branches of the peri-venous lymph trunks anastomose freely with the very irregular network of lymph vessels which is spread out between the pulmonary artery and vein. This network is formed by anastomosing vessels which connect the lymph vessels accompanying the lateral branches of the artery and vein with each other. At the root of the

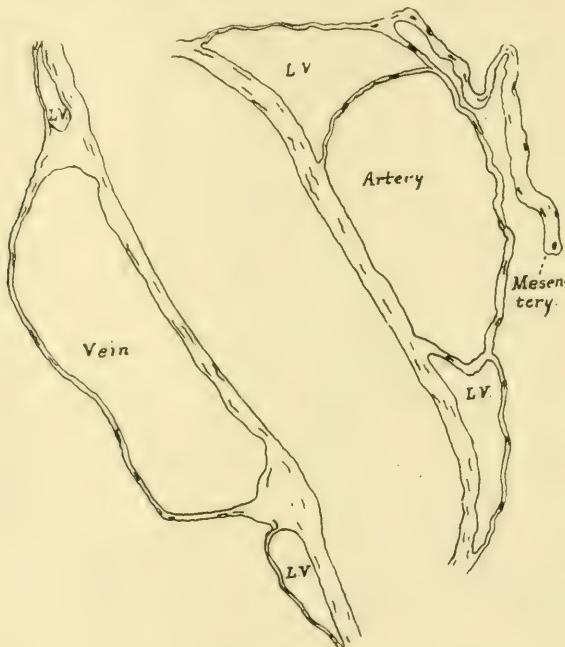


FIG. 2. Transverse sections through the pulmonary artery and vein, showing the position of the large lymph trunks (*L. V.*) and the attachment of the peritoneal fold which is cut through when injecting the lymph vessels. $\times 40$. (From Bulletin of the University of Wisconsin, No. 33, 1900.)

lungs the main peri-venous lymph trunks join a large lymph sinus which is situated on the ventral side of the stomach in the angle formed by the divergence of the lungs and through this with the network of lymph vessels situated on the ventral side of the stomach (Pl. I, Fig. 2).

The lymph vessels of the lung of *Necturus* like those of *Triton* and *Salamandra* (Suchard) are more superficially situated than the blood vessels. In transverse sections taken through the artery and vein, the position of the large lymph trunks is easily made out even though the

vessels are not injected (Fig. 2). In injected specimens the main blood vessels and their chief branches appear to be surrounded by a network of anastomosing lymph vessels. The lymph vessels themselves form a system of closed tubes. In none of the numerous preparations which I have made have I seen any evidence of so-called lymph capillaries, lymph spaces or lymph channels leading out from the lymph vessels. A distinct wall could be demonstrated for every vessel and the injection mass did not pass outside of this wall.

LYMPHATICS OF THE WALLS OF THE STOMACH.—The intimate relation between the lymph vessels of the lungs and those of the stomach renders it necessary to give a brief account of the latter. Reference to Plate I, Fig. 3 will give an idea of the exceeding richness of the plexus

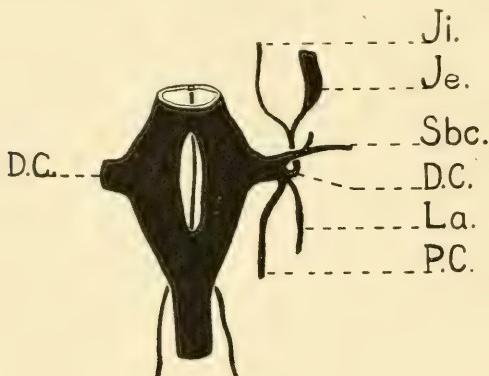


FIG. 3. Diagram of the duct of Cuvier and the principal venous trunks. *D. C.*, duct of Cuvier; *Ji.*, internal jugular; *Je.*, external jugular and jugular sinus; *Sbc.*, subclavian; *La.*, lateral; *P. C.*, postcardinal.

of lymph vessels present in the walls of the stomach. Along the dorsal side (Pl. I, Fig. 1) there is present a large and irregular sinus which communicates not only with the lymph vessels of the stomach and periarterial lymph vessels of the lungs but also with those belonging to the remaining viscera in the abdomen. On the ventral side (Pl. I, Fig. 2), as already mentioned, there is found in the angle formed by the diverging lungs a lymph sinus of considerable size and owing to the fact that there are no valves, the entire plexus can be easily injected.

CONNECTION OF LYMPH VESSELS WITH THE VEINS.—In order that the connection of the lymph vessels with the veins may be clearly understood it will be necessary to describe briefly the Ductus Cuvieri and the

venous trunks by which it is formed. Each Ductus Cuvieri (Fig. 3, *D. C.*) is formed by the union of the following veins:

jugular,
subclavian,
postcardinal,
lateral.

The jugular is the largest of the venous trunks and appears as a direct continuation of the Ductus Cuvieri. Its direction is at first dorsal, then curving anteriorly it passes above the subclavian to divide after a short distance into the external and internal jugulars (Fig. 3, *Ji., Je.*). Connected with the external jugular is a widened expansion, the jugular sinus (Fig. 3, *Je.*).

The subclavian (Fig. 3, *Sbc.*) joins the Ductus Cuvieri just internal to the jugular and on its ventral side. The postcardinal and lateral join the Ductus Cuvieri on the caudal side, the lateral being the more external (Fig. 3, *P. C., La.*). In some animals these last two veins unite just as they join the Ductus.

We have seen above how the lymph vessels of the lung are connected with the very rich network of lymph vessels in the walls of the stomach (Pl. I, Figs. 1, 2, and 3). Arising from the anterior and outer margin of this network of lymph vessels there is found on each side of the stomach a lymph trunk of moderate size which follows the course of the postcardinal vein until just before it joins the Ductus Cuvieri; it then passes dorsal to the postcardinal and lateral veins and enters sometimes directly into the Ductus Cuvieri, sometimes into the jugular (Pl. I, Fig. 3). Just before joining the vein this lymph trunk is joined by lymph vessels coming from the head and anterior extremity.

METHODS.—The technique of injecting the lymph vessels of the lung of *Necturus* is quite simple. The animal is killed with chloroform. If, on opening the abdomen, the lungs are not well distended, it facilitates the injection to insert a fine glass tube into the glottis and gently fill the lungs with air. The free tip of one of the lungs (I generally make use of the left lung) is grasped with a pair of broad pointed forceps and drawn away from the mid-line; this puts the peritoneal fold on the stretch. A nick is next made in this fold (Fig. 2), close to the artery, with a pair of sharp scissors, care being taken not to wound the artery itself. If the nick has been properly made a probe can now be introduced through this opening into one of the large lymph trunks which runs along the course of the artery. The cannula of a small syringe, which has been filled with a thin vermilion starch mass or Chinese ink rubbed up with normal salt solution, is pushed in beside the probe; the probe is now withdrawn and the cannula held in place between the thumb and finger of the left hand. The piston of the syringe is slowly

pushed in and the injecting mass can be seen running rapidly through the lymph vessels. The vessels are of considerable size, the mass flows easily, and, as there are no valves, but little pressure is necessary. The injection should always be made toward the head. This procedure should, with a little practice, give well filled lymph vessels in the lungs and also in the stomach. Warm masses do not give as good results as cold. I have frequently filled the lymph vessels of the lungs and stomach with a celloidin mass and have obtained very instructive preparations by digesting in pepsin.

In my hands granular injecting masses have given the best results, and thus one may avoid the uncertainty of, e. g., such fluids as aqueous solutions of Berlin or Prussian-blue. The lungs and stomach can be dissected out, hardened in alcohol, cleared in oil of clove, washed out with xylol, and mounted in balsam. By cutting the lungs and stomach open before mounting, the study of the preparation is made easier.

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EXPLANATION OF PLATES.

PLATE I.

FIG. 1. Dorso-lateral view of the stomach and right lung of *Necturus*. The heavily shaded vessel is the pulmonary artery; the lighter shaded vessels are the lymph vessels. The stomach was empty and contracted when the injection was made. Note the large sinus-like lymph trunk on the dorsal side of the stomach.

FIG. 2.—Ventral view of stomach and lungs of *Necturus*. Same preparation as Fig. 1. The heavily shaded vessels are the pulmonary veins; the lighter shaded vessels the lymph vessels. Note the sinus in the diverging angle of the lungs and the large lymph trunk on the mid-ventral surface of the stomach.

FIG. 3. Lymph vessels of the lung and stomach of *Necturus* and their connection with the veins. The shaded vessels are the lymph vessels; the veins are shown white. The plate is best seen from the side. The connecting vessel is indicated by the *. The left lung is reflected to the right and the stomach is partly dissected free.

PLATE II.

Lung of *Necturus* with the blood and lymph vessels injected. The lung was cut open, spread out flat, and mounted in balsam. A, artery; V, vein. Only the main branches of the artery and vein are shown. In two places the capillary network is indicated diagrammatically. The blood and lymph vessels were drawn by means of the camera lucida and show the exact relation of both sets of vessels.

THE VESSELS OF THE LUNG OF *NECTURUS MACULATUS*

PLATE I

W. S. MILLER

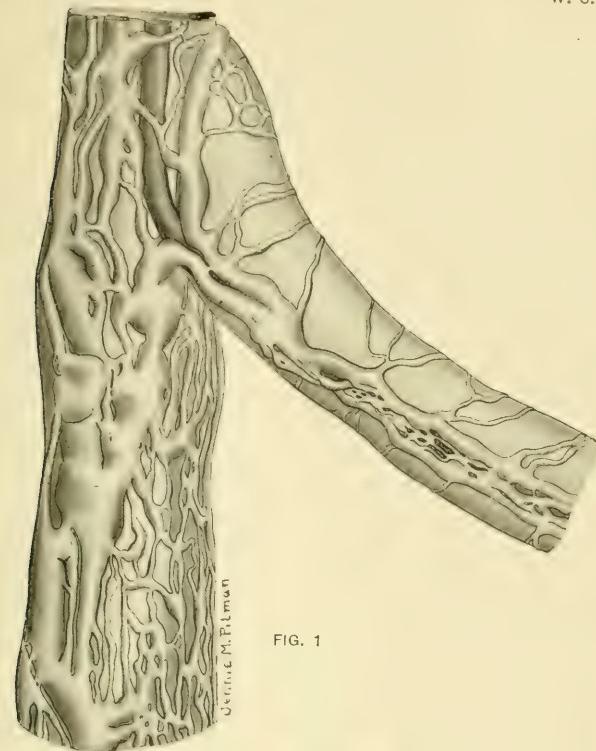


FIG. 1

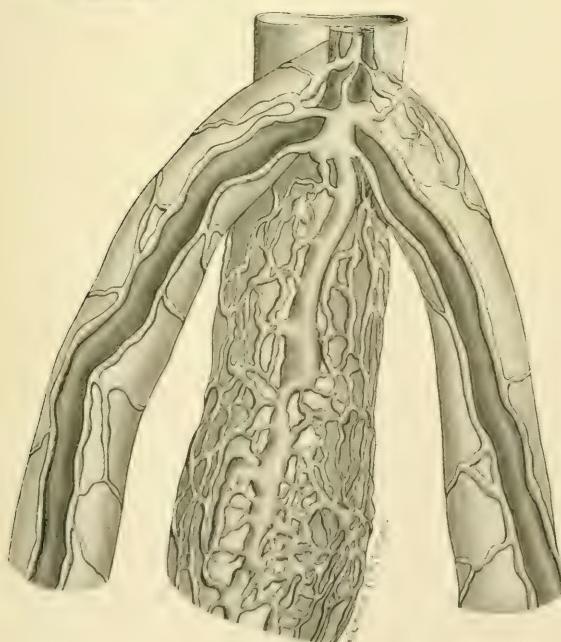


FIG. 2

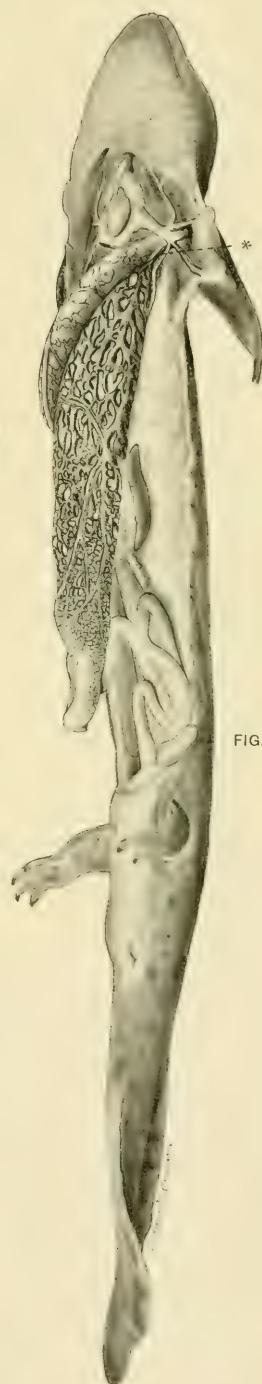
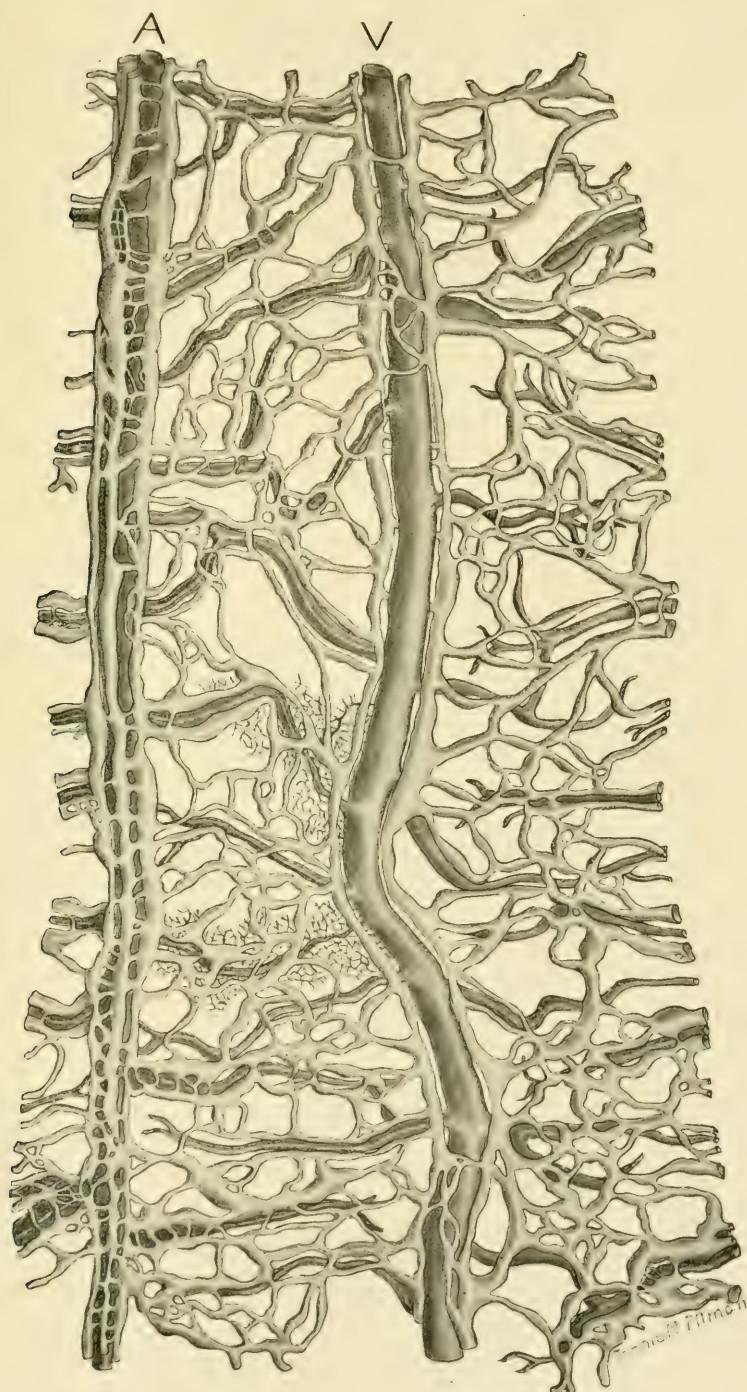


FIG. 3

THE VESSELS OF THE LUNG OF *NECTURUS MACULATUS*

PLATE II

W. S. MILLER



A CONTRIBUTION TO THE ANATOMY AND DEVELOPMENT OF THE VENOUS SYSTEM OF CHELONIA.

BY

FRANK A. STROMSTEN,

Fellow in Biology, Princeton University.¹

WITH 12 TEXT FIGURES.

The present series of observations on the anatomy and development of the venous system of turtles was made by the writer in the Morphological Laboratory of Princeton University. The material for the adult anatomy has been, for the most part, collected in the neighborhood of Princeton, New Jersey. All of the embryological material has, through the kindness of Professor McClure, been furnished by the Princeton embryological collection.

I take great pleasure in expressing here my deep obligations to Professor Charles F. W. McClure for his valuable suggestions and helpful criticism during the preparation of this paper. I am also indebted to Mr. Charles F. Sylvester for the preparation of some beautiful corrosions of the veins of the liver.

METHODS OF INVESTIGATION.

For the study of the adult venous system, about forty turtles of the more common species were dissected. These included the following forms:

CHELYDRIDÆ: *Chelydra serpentina* (L.).

KINOSTERNIDÆ: *Kinosternon pennsylvanicum* (Bosc.), *Aromochelys odoratus* (Latrelle).

EMYDIDÆ: *Chrysemys picta* (Hermann), *Clemmys (Chelopus) insculpta* (LeConte), *Clemmys guttatus* (Schneider), *Terrapene carolina* (L.).

The turtles were killed with chloral hydrate and injected through the left abdominal vein. Chloral hydrate was used in preference to either chloroform or ether because it leaves the animal in an extended con-

¹ Presented to the Faculty of Princeton University for the degree of D. Sc.
AMERICAN JOURNAL OF ANATOMY.—VOL. IV.

dition and to a large extent gets rid of the muscular contractions. The best results were obtained when the turtle was killed several days before the injection was made. A gelatin injecting mass was used in most cases. In order to prevent the cooling of the gelatin before all the veins were filled, the specimens were placed in warm water several minutes before injecting. This is not necessary, however, if potassium iodide is used to lower the melting point of the gelatin. For the purpose of studying more exactly the relations of the veins in the liver and the kidneys, some of the turtles were injected with Huntington's, 97, wax injecting mass and corroded with concentrated commercial hydrochloric acid. By injecting the liver through the precava with one color and through the abdominal vein with another, it is possible to distinguish the advehent from the revehent systems even to their finest branches. A few of the larger snapping turtles were injected with the common starch injecting mass.

About fifty turtle embryos were studied for the development of the veins. *Kinosternon pennsylvanicum* was the species chosen for embryological investigation because it was possible to obtain a more complete series of this than of any other form. The more important stages were also studied in *Chrysemys picta*. For purposes of comparison several embryos of the common lizard, *Sceloporus undulatus*, as well as chick embryos of various stages, were examined.

Most of the material had been fixed in picro-sublimate or picro-nitric. The picro-sublimate material was, as a rule, satisfactory both as to fixation and for the subsequent staining reactions. On the other hand, not much can be said in favor of picro-nitric either for fixing the tissues, or for preparing them for the stain. The embryos were dehydrated, cleared, and embedded in paraffin according to the usual methods, and serial sections were cut about 20μ thick. Several methods of staining were tried, but the best results were obtained with Delafield's haematoxylin and pieric acid. After picro-sublimate fixation this combination differentiates the blood vessels very clearly. Frontal reconstructions, in whole or in part, were made of the venous system of all the embryos studied. Wax reconstructions were made wherever deemed necessary.

HISTORICAL SKETCH.

The literature dealing with the embryology of the venous system of Chelonia is very fragmentary. Rathke, 48, and Agassiz, 57, are the only investigators that have hitherto made any attempt whatsoever to even superficially sketch the development of the veins of this interesting reptilian form. Agassiz gives as a reason that so little is known about

the embryology of turtles the fact that "In the Old World, no turtles are found in the immediate vicinity of the great centers of study."

Rathke, 48, in his monograph, "*Ueber die Entwicklung der Schildkroeten*," merely mentions the presence of some of the larger veins in a few of the earlier stages. His older embryos were so poorly fixed that it was not possible for him to trace the advanced stages of venous development. Agassiz's remark that, "It is felt, on almost every page of his work, that he labored under a scarcity of materials which constantly impeded his progress," is particularly true in respect to his treatment of the venous system.

About ten years later, Agassiz, 57, undertook to "write anew the embryology of this order of reptiles," that he might continue the work which Rathke was unable to complete. His investigations, which are published in a large volume of nearly two hundred quarto pages, together with thirty-two plates, are concerned chiefly with the study of the embryo as a whole without the aid of serial sections. Hence, almost no attention is paid to the development of the intra-embryonic veins, although considerable space is devoted to the consideration of the vitelline circulation.

It is thus seen that no serious attempt has hitherto been made to give a complete history of the development of the veins of turtles. On the other hand, very careful and accurate observations have been made by a number of investigators on the embryology of the veins of snakes and lizards. Rathke, 39, Hoffmann, 90, Hochstetter, 93, Grosser and Brezina, 95, and Choronshitzky, 99, have investigated in whole or in part the development of the venous system in these reptiles so that our knowledge here is quite complete.

The purpose of the present paper is: (1) to confirm the work already done on reptiles by other writers, and to furnish another link in the comparative embryology of the venous system of amniota; (2) to investigate somewhat more in detail than has hitherto been done the changes which the umbilical veins undergo, and their ultimate relations to the abdominal veins. As a basis, therefore, for comparing the various stages of venous development in Chelonia with those of other reptiles, a brief résumé of the chief results of Hochstetter's (93 and 03) papers is given below.

DEVELOPMENT OF THE VEINS OF LACERTA AND TROPIDONOTUS.

According to Hochstetter, the first veins to appear in reptiles are the omphalo-mesenteries. These are shortly followed by the precardinals, the postcardinals, and the umbilicals.

The transformation of the paired omphalo-mesenteric veins into a single vessel to form the anlage of the portal vein.—The two omphalo-mesenteric veins fuse dorsal to the intestine just caudad of the first anlage of the pancreas to form the dorsal anastomosis and again further back ventral to the intestine to form the posterior ventral anastomosis. A ring is thus formed around the intestine which, however, is soon broken by the atrophy of that part formed by the right vein. In the meantime both omphalo-mesenteric veins craniad of the dorsal anastomosis are more or less broken up into smaller vessels by the liver tubules. In snakes, the left omphalo-mesenteric vein loses its connection with the sinus venosus before the posterior ventral anastomosis takes place, while, on the other hand, in lizards this connection is retained until the formation of the ring mentioned above is completed. In both reptiles it is the right vein that persists in front of the dorsal anastomosis and the left vein behind it. There is thus formed a single vessel, arising from the vitelline veins at the umbilicus, which takes a spiral course around the intestine and enters the right half of the liver where it breaks up to form the hepatic network. The vein thus formed constitutes the anlage of the portal vein. The cardiac end of the right omphalo-mesenteric vein is retained as a part of the *right hepatic revehent vein*.

The formation of the postcava, and the reversal of the primitive renal portal system.—The revehent veins of the primitive renal portal system of reptiles are represented by the postcardinals. In lizards, the postcardinal veins arise in the caudal region of the body and extend forward dorsal to the mesonephric ducts to join the precardinals and with the latter form the ducts of Cuvier. They are broken up near the cranial ends of the mesonephroi into a number of broad blood sinuses (*Bluträumen*) and lose their dorsal position. In snakes, the postcardinals are connected with the caudal vein from the first and do not lose their integrity as distinct vessels at their cranial ends.

The advehent veins are formed by the bifurcation of the caudal vein. The two branches thus formed extend forward along the ventromedial border of the mesonephroi and appear to end blindly a short distance in front of the origin of the omphalo-mesenteric artery. As a matter of fact, however, they are connected with the postcardinals by means of the mesonephric network. All of the blood from the caudal vein must, therefore, in order to reach the heart, stream through the mesonephroi.

The primitive renal portal system just described soon undergoes a complete reversal. One of the most important events connected with this change is the formation of the postcava. This is brought about in the following manner: first, the two branches of the caudal vein fuse

just craniad of the origin of the omphalo-mesenteric artery to form an unpaired stem. A short branch is then formed in the caval mesentery which connects the unpaired stem just mentioned with the right hepatic revehent vein in lizards, but with the common hepatic vein in snakes. A continuous path is thus formed from the caudal vein direct to the heart, and the result is that most of the blood from the hinder parts of the body now reaches the heart by this route. The new path thus formed is the first anlage of the postcava.

Craniad of the origin of the omphalo-mesenteric artery the postcava is an unpaired vessel formed from the following parts: the fused portion of the two branches of the caudal vein, the new formation in the caval mesentery, sinusoids of the liver, and the right hepatic revehent vein (lizard), or the common hepatic vein (snakes). Caudad, the postcava is paired and is formed by the two branches of the caudal vein. The postcava loses its connection with the caudal vein, however, while at the same time a strong anastomosis is formed between the caudal vein and the postcardinals. It thus appears that while the postcava is still connected with the caudal vein, it may function for a short time both as a revehent and as an advehent vein to the mesonephroi.

Shortly after the postcardinals have joined the caudal vein they lose their connection with the sinus venosus and the reversal of the primitive portal system of the mesonephroi is thus completed. The postcardinal veins which now receive the blood from the caudal vein act as advehent veins, while the postcava with its branches becomes the revehent system.

Further changes in the postcava.—During the time the above changes are taking place, the two branches of the postcava (original branches of the caudal vein) have fused just caudad of the origin of the omphalo-mesenteric artery so that this artery appears to pass through a foramen in the unpaired postcava. The unpaired postcava craniad of the omphalo-mesenteric artery receives a branch on each side from the cranial regions of the mesonephroi. The left cranial mesonephric branch then begins to split away from the main stem backward as far as the origin of the omphalo-mesenteric artery so that finally this artery no longer passes through the postcava, but to the left side of it. The postcava now extends as an unpaired vessel from the sinus venosus to the origin of the omphalo-mesenteric artery, immediately caudad of which it divides into two branches. The right branch passes backward as a direct continuation of the postcava, while the other branch bends sharply to the left behind the origin of the omphalo-mesenteric artery, and then proceeds backward. The left cranial mesonephric vein opens

into this left branch of the postcava, while the right cranial mesonephric vein opens directly into the main stem of the postcava.

Development of the posterior vertebral veins.—The degeneration of the cranial portions of the mesonephroi is accompanied by the gradual atrophy of the postcardinal veins from before backward. As these veins degenerate, their dorsal intersegmental branches fuse ventral to the ribs to form a pair of longitudinally running vessels, the posterior vertebral veins of the adult. They arise in the caudal region of the body and extend along its dorsal wall, one on each side of the vertebral column, to open into the subclavian veins. A connection between the liver circulation and the posterior vertebral veins is also made by means of two or three branches.

The posterior vertebral veins are, at first, symmetrically developed on each side of the body in snakes just as they are in the lizards. In snakes, however, anastomoses early develop between this pair of veins and the veins of the oesophagus, stomach, and portal system, which soon result in the degeneration of the vertebral veins, so that they are represented in the adult system only as fine anastomoses between the intercostal veins. According to Rathke, they may in some cases disappear altogether. Hochstetter attributes the degeneration of these veins to the peculiar locomotion of snakes.

Advehent veins of the permanent kidneys.—The caudal portion of the postcardinal veins in snakes, as well as in lizards, is still retained in the adult system as the advehent veins of the permanent kidneys. Since the permanent kidneys arise dorsal to the mesonephroi, the persisting portions of the postcardinal veins are found on their ventral surfaces, but still retain their original position dorsal to the mesonephric ducts.

The umbilical veins and their transformations.—The umbilical veins of a very young embryo of the lizard arise in the allantois and enter the body through the umbilicus. In the umbilicus they unite to form a single vein, but separate again on entering the body. They extend forward in the body-wall and open into the sinus venosus together with the omphalo-mesenteric vein of the same side. At the umbilicus they receive a pair of small veins which extend backward along the ventral body-wall to join the postcardinals. This small pair of veins constitutes the posterior anlage of the abdominal veins. In a later stage than this just described, the left umbilical vein connects with the hepatic network. Soon a direct channel is formed through the liver between the left umbilical vein and the hepatic revehent vein. The left umbilical vein completely degenerates craniad of the point where it enters the liver so that all of the blood from this vein must pass through the liver in order

to reach the heart. Later, the portal vein joins the hepatic portion of the left umbilical, so that there is a direct channel from the portal to the hepatic revehent vein. The right umbilical vein does not enter the liver in the lizards, but in snakes it very early fuses with the right omphalo-mesenteric vein to form a common stem and thus comes to lie within the liver for a considerable distance. Another important difference between the snakes and lizards is found in the fact that in the former there is no direct connection between the left umbilical vein and the portal, as there is, as we have seen, in the lizards. In both of these reptiles, however, the abdominal portion of the right umbilical vein completely degenerates, while the same portion of the left vein continues to increase in size for some time although it, too, apparently degenerates after birth.

The abdominal veins.—The abdominal veins are developed, as has already been stated, from a pair of small vessels which extend along the ventral abdominal wall and connect the umbilical veins with the post-cardinals. Later they fuse in the mid-line and connect with the portal vein. As soon as this latter connection is made the connection with the umbilicals is lost. According to Hochstetter, the umbilical veins do not enter into the formation of the abdominal veins in lizards and snakes.

DEVELOPMENT OF THE VEINS OF KINOSTERNON PENNSYLVANICUM.

Unless otherwise stated, the following description will be based entirely on the embryos of *Kinosternon pennsylvanicum*. Owing to the relatively advanced stage of development of the youngest embryo studied, it is not possible to describe the earliest appearance of some of the veins. Furthermore, no attempt will be made to trace the development of veins not directly connected with one or the other of the portal systems. The plan of the present paper will be to take up first a study of the anatomy and development of the hepatic portal system, and then a similar study of the renal portal system. It is not the purpose of the writer to give at this time a detailed comparative description of the adult venous system. For this reason only those veins are mentioned whose development is to be more or less carefully traced.

HEPATIC PORTAL SYSTEM.

VEINS OF ADULT KINOSTERNON.—Fig. 1 represents the general arrangement of the liver veins of *Kinosternon pennsylvanicum*. As shown by the figure, the hepatic venous system of turtles is made up of two entirely distinct sets of veins, the revehent and the advehent veins.

The revehent system consists of the postcava, the left hepatic revehent vein, and several smaller veins, all of which open separately into the sinus venosus.

The largest of the revehent veins is the hepatic postcava. It passes directly through the right superior lobe of the liver and, after receiving a number of branches from this organ, opens into the right side of the sinus venosus. The largest of its hepatic tributaries is the right hepatic vein which usually opens into the postcava where the latter joins the sinus venosus.

The other large vein which opens into the sinus venosus is the left hepatic revehent vein.

Besides these two large veins, a number of smaller ones open directly

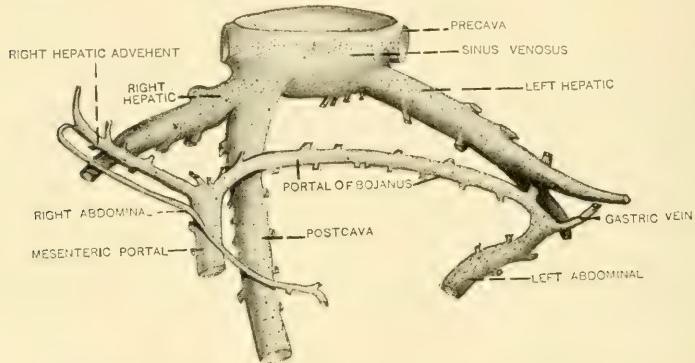


FIG. 1. Hepatic portal system of *Kinosternon pennsylvanicum*. Ventral view.

into the sinus venosus from the liver. The number of these is not constant, but depends, as we shall see when we come to study their development, upon the relative backward extension of the venous sinus.

The advehent system consists of the two abdominal and the portal veins.

The abdominal veins (see also Fig. 10) arise as branches of the circumflex iliacs of each side and extend mediad and craniad for a short distance along the cranial border of the ventral segment of the pelvic girdle. Just in front of the apex of the triangle formed by the union of the two pubic bones, the two abdominals are connected by a short anastomosing branch. From this point they continue forward along the abdominal wall as separate vessels and then enter the liver.

The left abdominal vein is much larger than the right. It passes dorsad through the left inferior lobe of the liver to its gastro-duodenal

surface where it unites with a vein from the lesser curvature of the stomach, the gastric vein, to form the portal vein of Bojanus. (*Bojanus, Anatome Testudinis Europæa.* Tab. XXV, Fig. 128, sz.)

The right abdominal vein passes through the right inferior lobe of the liver and may open into portal vein of Bojanus, the mesenteric portal, or, as in Fig. 1, into a large lateral branch of the latter.

The portal vein of Bojanus (which in the adult is really the direct continuation of the left abdominal vein) arises, as stated above, from the union of the left abdominal with the much smaller gastric vein. It passes directly across the body, from left to right, embedded in a groove on the ventral surface of the "isthmus" of the liver, apparently increasing very gradually in calibre as it proceeds, and finally joins the mesenteric portal where the latter enters the liver. It receives during its course a number of tributaries from the duodenum, pancreas, and liver. The largest of these branches comes from the dorsal region of the left inferior lobe of the liver, and probably functions as the chief advehent vein for that region.

The mesenteric portal vein arises in the mesentery of the intestines and enters the right half of the liver where it breaks up into the hepatic venous network. The chief branches of the mesenteric portal are the following veins: the descending colic, the ileo-colic, the intestinal, the splenic, the posterior pancreatic, and the portal vein of Bojanus. Within the liver it gives off a number of advehent branches, the largest of which usually receives the right abdominal vein.

DEVELOPMENT OF THE VEINS OF THE HEPATIC PORTAL SYSTEM IN KINOSTERNON PENNSYLVANICUM.

The embryonic veins to be considered in this connection are: the omphalo-mesenteric, or vitelline veins, and the umbilicals, or allantoic veins.

In the youngest stage studied (7.4 mm. crown-rump measurement around the curvature of the body),² the anterior limbs are just beginning to make their appearance as a low ridge involving several muscle segments on each side of the body. The lungs are not yet indicated. The liver is represented by a few tubules ventral to and on the right side of the intestine. The first anlage of the pancreas has just been split off as a simple tube from the dorsal side of the flat mid-gut. The venous system is represented by the following five pairs of veins: (1) the

² All measurements were made in this manner.

omphalo-mesenteries, (2) the precardinals, (3) the postcardinals, (4) the umbilicals, and (5) the subcardinals of Lewis, **o2.**

The Transformations of the Omphalo-Mesenterics to form the Mesenteric Portal and the Right Hepatic Vein.

The omphalo-mesenteric veins in an embryo of about 7.4 mm.—In this stage the omphalo-mesenteric veins are represented by a pair of vessels arising in a network of the yolk sac and extending forward along the dorsal wall of the mid-gut near the outer border of the intestinal epithelium. Craniad, they gradually approach each other and finally fuse immediately caudad of the first anlage of the pancreas to form the dorsal anastomosis. Both veins are about equal in size at this point, but further caudad the left vein is somewhat larger. Craniad of the dorsal anastomosis, both veins enter the liver. The left vein passes around the border of the liver and is not broken up by the hepatic tubules. It opens into the sinus venosus close to the umbilical of the same side. The right vein, on the other hand, soon after entering the liver is more or less broken up into a number of smaller vessels which unite again at the cranial end of the liver and open into the sinus venosus as a single vessel.

In an embryo of nearly the same measurement, but in which the first indications of the lungs appear, the right omphalo-mesenteric vein is further broken up to form a rete mirabile. This breaking up continues to such an extent that all of the blood from the right vein must pass through a network of sinusoids in order to reach the heart. In the meantime the left vein also begins to break up somewhat and there is established a sinusoidal connection between the two halves of the liver. The clogging up, as it were, of the entire hepatic portion of the right omphalo-mesenteric vein by the invasion of the liver tubules causes a large portion of the blood from this vein to stream through the sinusoids to the much freer channel of the left vein. This eventually results in the formation of an anastomosing branch through the liver connecting the two omphalo-mesenteric veins (Fig. 2). The branch thus formed arises at about the point where the right vein begins to break up and passes diagonally through the liver to the left vein, ventral to the intestine. The blood of the right vein that does not pass through this anastomosis still streams through the sinusoids and is collected at the cranial end of the liver by two or three small vessels and thus carried to the sinus venosus.

Thus we find in turtles as well as in snakes and lizards that a double anastomosis is very early formed between the two omphalo-mesenteric

veins. Usually the dorsal anastomosis is the first connection formed between these two veins and then later a ventral anastomosis is formed craniad of this through the liver. Choronzitzky, 190, however, has found a curious variation from this in *Anguis fragilis*. In this reptile, according to that investigator, a ventral anastomosis is formed through the liver before the dorsal anastomosis and is to a great extent the cause of the latter, as is seen by the following extract from his paper on "Die Entstehung d. Milz, Leber, Gallenblase, Bauchspeicheldrüse, etc." (Anatomische Hefte, XIII, S. 572):

"Die zwischen der queren Anastomose und hinterem Ende des Sinus venosus befindlichen und in die Leberanlage eingeschlossenen Teile der beiden Venae omph.-mesentericae wurden unterdessen durch die starke

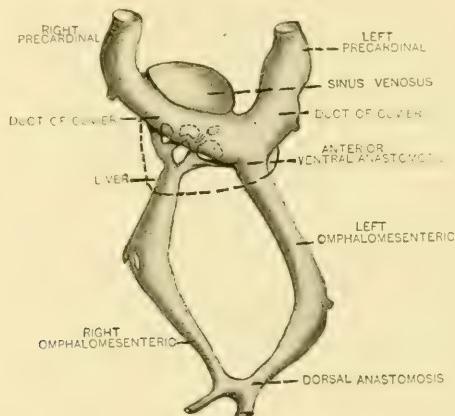


FIG. 2. Frontal reconstruction of the liver veins of a 7.5 mm. embryo of *Kinosternon pennsylvanicum*. Ventral view.

Zerklüften ihrer Wände in ein doppeltes Rete mirabile umgewandelt, wobei aber von der rechten Vena omph.-mes. noch ein die Leberanlage kaudo-kranial durchbohrender starker Ast nachblieb, während die linke Vena omph.-mes. sich in ganz schmächtige Lebervenen aufgelöst und von der queren Anastomose abgetrennt hat. Der hinter dieser Anastomose befindliche Abschnitt der Vena omph.-mes. sinistra muss jetzt sein Blut in die gen. Anastomose ergießen, damit es durch den in die Leberanlage eingeschlossenen Teil der Vena omph.-mes. dextra zum Herzen gelangen kann. Aber die quere Anastomose wird, . . . stark durch die beiden Lebergänge komprimiert. Das Blut muss sich einen anderen Ausweg suchen und findet einen solchen in der Gestalt einer anderen Anastomose, welche weiter hinten liegt und, die dorsale Darm-

wand umbiegend, beide Venae omph.-mes. vereinigt." (Dorsal anastomosis.)

This will not account for the formation of the dorsal anastomosis in turtles. In the first place, the dorsal anastomosis is the first connection formed between the two omphalo-mesenteric veins. Also, it is formed at a time when the left vein has reached its maximum of development and before the right vein is greatly broken up in the liver. In *Kinosternon*, the formation of the dorsal anastomosis seems to be almost entirely due to the pinching together of the dorsal wall of the mid-gut to form the dorsal anlage of the pancreas. The two veins are thus brought so near each other that even a slight obstruction of the blood current might cause the thin parting walls to break and thus establish a connection

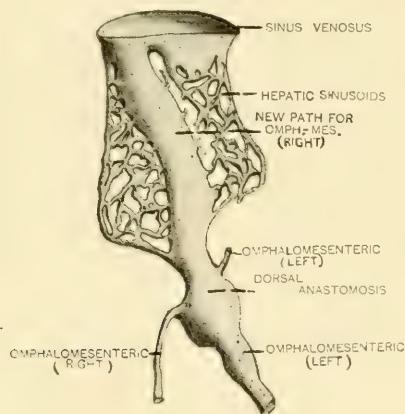


FIG. 3. Frontal reconstruction of the liver veins of an 8 mm. embryo of *Kinosternon pennsylvanicum*. Ventral view.

between them. In other words, it may be due not so much to the necessity for a connecting path between the two veins that the dorsal anastomosis is formed, as it is to the position of these veins on the dorsal wall of the mid-gut previous to the formation of the anlage of the pancreas.

This brings us to an interesting comparison of the varying lengths of time which the left vein retains its connection with the sinus venosus in the different reptiles. In *Anguis*, as we have seen, this connection is lost before the dorsal anastomosis is made. In *Tropidonotus* and *Kinosternon*, on the other hand, it is retained until after the formation of the dorsal anastomosis but degenerates before the two omphalo-mesenteric veins fuse further back to form the so-called posterior ring around the intestine. In *Lacerta*, this connection with the sinus venosus per-

sists still longer, and is present for a short time after the posterior ring is formed.

The rapid development of the liver gradually forces the stomach, together with the left omphalo-mesenteric vein, from its original median position. At the same time this vein becomes more and more broken up into a rete mirabile. This change in position, as well as the breaking up of the left omphalo-mesenteric vein, causes it to be a less favorable route for the blood to reach the heart than the sinusoids of the right half of the liver. As a result, we find in an 8 mm. embryo (Fig. 3) that a new path has been formed for the right vein which soon serves to carry most of the blood from the omphalo-mesenteric veins to the heart. The left omphalo-mesenteric vein then begins to degenerate and eventually loses its connection with the sinus venosus. In the meantime, the anastomosing branch through the liver connecting the two omphalo-mesenteric veins has been gradually breaking up into sinusoids so that later it becomes entirely lost in the hepatic network. All of the blood from the liver must now enter the sinus venosus through the newly formed hepatic portion of the right omphalo-mesenteric vein.

The omphalo-mesenteric veins of an embryo of about 10 mm.—The two omphalo-mesenteric veins now fuse ventral to the intestine some distance caudad of the dorsal anastomosis to form the posterior venous ring around the intestine (Fig. 4). The left vein craniad of the dorsal anastomosis is now represented by a very slender branch which passes forward and downward in the wall of the intestine without entering the liver. The right omphalo-mesenteric vein splits soon after entering the liver into two branches of nearly equal size which, after passing forward a short distance, unite again to form a single vessel. Since these two branches are to form the right hepatic rete and the mesenteric portal veins, while the common stem formed by their union craniad is to be the common hepatic vein, they have been designated by these names in Fig. 4.

Just as the right omphalo-mesenteric vein enters the liver, it receives a small vein from the ventral surface of the stomach, the gastric vein of Fig. 4. As this vein is to play a somewhat important rôle in the formation of the portal vein of Bojanus, it is advisable at this point to notice briefly its earlier development.

The gastric vein is first definitely seen at the time when the ventral anastomosis between the two omphalo-mesenteric veins is formed through the liver. It then is a very short branch, situated in the ventral mesentery of the stomach anlage and opens into the anterior ventral anastomosis. Later, as the liver increases in size, the vein becomes longer, and

when the ventral anastomosis is destroyed it opens into the right omphalo-mesenteric vein. During further development, as we shall see, it comes to connect with the mesenteric portal vein. Finally, the left umbilical vein unites with the gastric vein and their common trunk forms the portal vein of Bojanus. In the meantime the twisting of the stomach brings the gastric vein to lie on the lesser curvature of that organ.

In the 10 mm. stage, we find the first definite indications of a connection being formed between the right hepatic and the right sub-

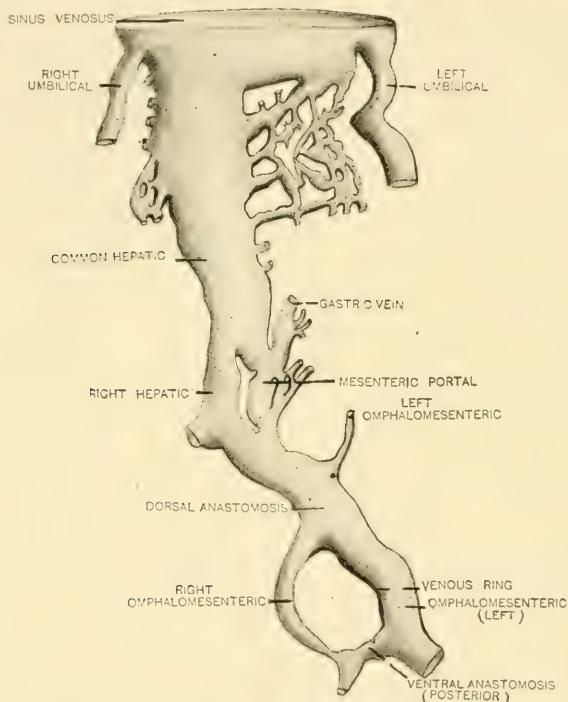


FIG. 4. Frontal reconstruction of the liver veins of a 10 mm. embryo of *Kinosternon pennsylvanicum*. Ventral view.

cardinal vein (not shown in Fig. 4). A small vein is formed in the ventral border of the caval mesentery which extends from the right subcardinal vein to the dorsal border of the liver where it apparently breaks up into a number of finer vessels. Whether these make connections with the liver or not, it is impossible to determine with certainty.

The omphalo-mesenteric veins of an embryo of about 11 mm.—Fig. 5 is a reconstruction of the liver veins of this stage. For sake of clearness the smaller branches and the sinusoids are omitted. The posterior ring

around the intestine is now destroyed by the atrophy of that part formed by the right omphalo-mesenteric vein. Thus we have the unpaired anlage of the mesenteric portal vein formed from the originally paired omphalo-mesenterics.

The splitting of the right omphalo-mesenteric vein to form the right

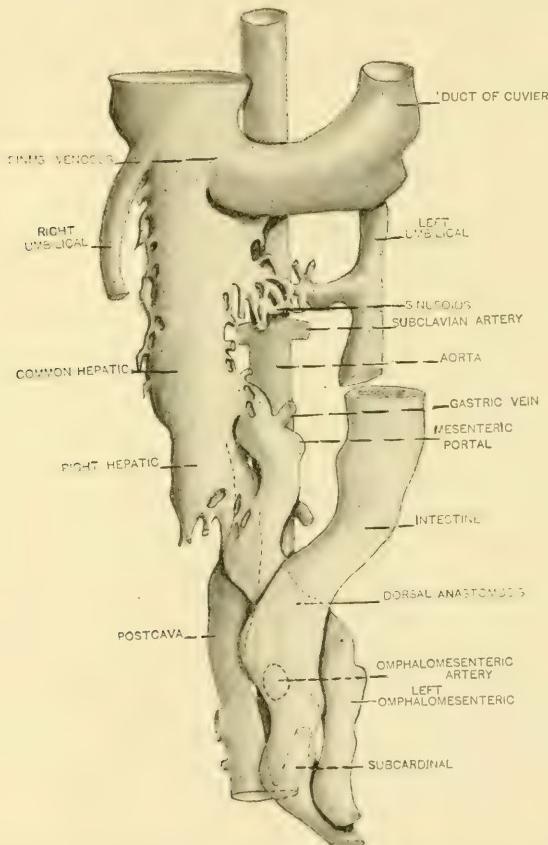


FIG. 5.—Frontal reconstruction of the liver veins of an 11 mm. embryo of *Kinosternon pennsylvanicum*. Ventral view.

hepatic and the hepatic portion of the mesenteric portal, which was begun in the last stage, is still more extensive in this stage. While, at first, the right hepatic and the mesenteric portal veins are united both cranially and caudally, they now become more and more separated through the breaking up of these connections into sinusoids, as shown in Fig. 5, in which this process has already begun.

The omphalo-mesenteric veins of an embryo of about 12 mm.—In this stage the separation of the right hepatic from the mesenteric portal is still more complete. The original connection between the two veins is now almost entirely reduced to sinusoids (not shown in Fig. 6). There still remains, however, one, sometimes two, direct anastomosing branches which may represent the remains of the original connections. These, when present, occur just after the mesenteric portal enters the liver.

We find at this stage, in addition to the original connections between these two veins, a series of three or four anastomosing branches that

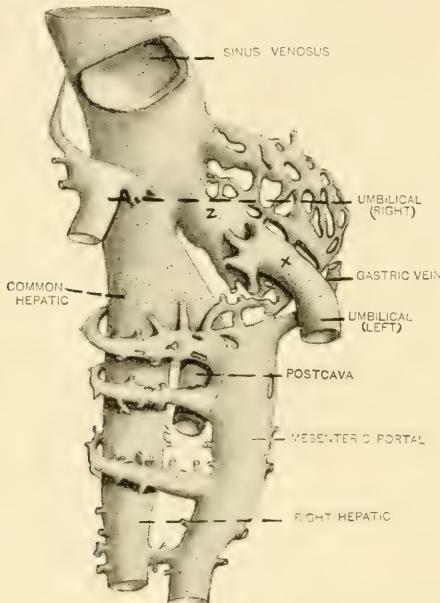


FIG. 6. Frontal reconstruction of the liver veins of a 12 mm. embryo of *Kinosternon pennsylvanicum*. Ventral view.

appear to be new formations. As shown by Figs. 6 and 7 "A," they take a somewhat curved course through the liver and open into the dorsal side of the right hepatic vein. They appear to be formed through the fusion of some of the sinusoids ventral to, and on the right side of, the right hepatic vein. The first indications of these curved veins appear in the 11 mm. stage.

In the 12 mm. stage these curved veins have reached their maximum of development. The first one to be given off from the mesenteric portal on entering the liver is found a short distance caudad of the gall bladder, while the other two pass dorsal to it, as in Fig. 7. When the fourth is

present it is found further craniad and opens into the common hepatic vein. As shown by Fig. 6, one of these branches is given off from the mesenteric portal almost directly opposite the opening of the gastric vein. This branch persists in the adult form where it functions as the chief lateral branch of the mesenteric portal, the right hepatic advehent branch of Fig. 1. It is possible that the other branches are also retained, in part at least, as branches of the mesenteric portal of the adult, but it is not possible to trace their subsequent development with certainty.

The right omphalo-mesenteric vein has split still further craniad than in the previous stage, so that, as shown in Fig. 6, there is a small portion

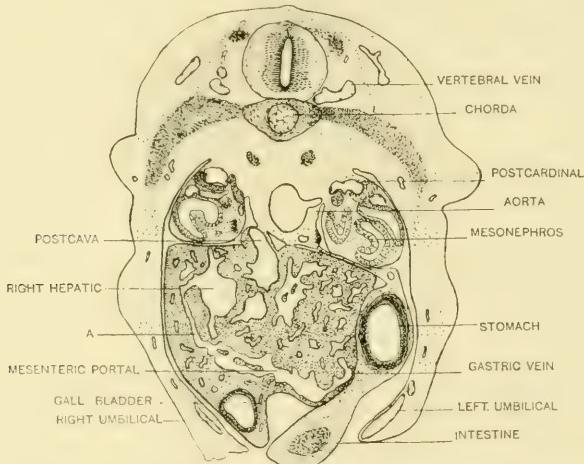


FIG. 7. Cross-section of a 12 mm. embryo of *Kinosternon pennsylvanicum*, at the level where the postcava enters the liver, showing the curved anastomosis (A) between the hepatic and mesenteric portal veins and the opening of the gastric vein into the mesenteric portal. The portal vein of Bojanus is not yet formed.

(Z) of the left branch next to the common hepatic vein that is not yet broken up into sinusoids. A part of this short, wide stem is retained in the adult as the terminal portion of the left hepatic revehent vein, the other part of this hepatic vein is, as we shall see, developed from the umbilical vein. A part of the left revehent vein, therefore, as well as nearly the entire anlage of the right hepatic vein, is split off from the right omphalo-mesenteric vein.

Further changes in the derivatives of the omphalo-mesenterics.—The right hepatic and the mesenteric portal veins are becoming more and more separated. The curved anastomosing branches which were so prominent in the 12 mm. stage begin to break up in the 13 mm. stage at the

point where they open into the hepatic vein, and in a 14 mm. embryo they no longer connect with the right hepatic except through the general sinusoidal network of the liver. In the 15 mm. stage the derivatives of the omphalo-mesenteric veins have practically assumed their adult relations.

THE UMBILICAL VEINS. THE FORMATION OF THE RIGHT AND LEFT ABDOMINAL VEINS AND THE LEFT HEPATIC VEIN.

It is not possible from the material at hand to determine anything definite about the origin of the umbilical veins in turtles. Dr. Frederic T. Lewis, who has made a very careful study of the early development of the umbilical veins in the rabbit, has very kindly sent the writer a number of drawings and sketches (not yet published) which undoubtedly show that, in the rabbit at least, the umbilical veins appear earlier than the postcardinals and arise in part from the vitelline veins and in part from the intersegmental arteries, thus: "The vitelline vein sends sprouts forward and backward in the somatopleure. The forward branch becomes the anterior cardinal vein; the posterior branch, incorporating in its progress prolongations of the intersegmental arteries, becomes the umbilical vein." (Lewis: Am. Jour. Anat., 1904, Vol. III, p. XII.)

In the 7.4 mm. embryo of the turtle, as we have already seen, the postcardinals and umbilicals are both present. The umbilicals are, however, much smaller than the postcardinals and are connected with them, especially near their cardiac ends, and through the region of the anterior limb buds, by a large number of cross branches. Caudally, the umbilical veins cannot be followed with certainty.

In the 9 mm.-embryo, however, they become very large and may easily be followed along their entire length. At this stage (9 mm.) they are of nearly equal size. They arise in the allantois and extend along the body wall near the somato-amniotic angle and open into the sinus venosus close to, or in common with, the omphalo-mesenteric vein of the same side. In most embryos of this age the right umbilical vein appears to open into the right omphalo-mesenteric vein, while the left opens into the sinus venosus some distance from the omphalo-mesenteric vein of that side. Both umbilicals are still somewhat connected with the postcardinals especially through the limb anlagen.

Umbilical veins of a 10 mm. embryo.—The umbilical veins are now increasing very rapidly in size and importance. They begin, at about this time, a series of changes which is destined to play an important rôle in the development of the portal system of the liver. This is brought about chiefly by the separation of the pericardial cavity.

In the 7.4 mm. embryo, the pericardial cavity is separated from the pleuro-peritoneal cavity by the septum transversum. The ducts of Cuvier and the umbilical veins reach the sinus venosus through the lateral portions of this septum, that is, by the lateral mesocardes of Koelliker. Later, two folds are developed in the body-wall just caudad of the septum transversum. These folds grow mediad, ventrad, and caudad, separating the liver from the heart from before backward. The umbilical veins lie in the body-wall just at the region where this ingrowth of connective tissue takes place. They are, therefore, carried mediad with the ingrowth of these folds, beginning at the point where these veins open into the sinus venosus and gradually extending caudad. In the present stage (10 mm.) the left umbilical vein is just beginning to show a slight deviation mediad, while the right is yet unaffected.

The umbilical veins now no longer connect directly with the postcardinal veins in the pelvic regions, but indirectly by means of a small pair of veins which are formed on the ventral abdominal walls caudal to the stalk of the allantois. In this stage, these veins are still very short. They form the posterior anlagen of the abdominal veins of the adult, and are designated in this paper as the postabdominals.

Umbilical veins connect with the hepatic circulation. Embryo of about 11 mm.—The left umbilical vein has now been carried so far mediad that for several sections it comes to lie between the heart and the liver, and instead of opening into the sinus venosus laterally as at first, it now opens into its dorsal side (Fig. 5). Being thus squeezed in, as it were, between the liver and the heart, the vein becomes more or less flattened in this region. Instead of being able to increase as rapidly in size as the caudal portion, it really gradually becomes smaller. The rapid development of the allantois at this time results in an enormous increase in the size of the umbilicals. It therefore becomes necessary for this ever-increasing amount of blood to find some way to the heart other than the narrow cardiac end of the left umbilical vein. It so happens that at the point where the left vein leaves the body-wall to enter the pericardium, it comes to lie very close to the ventral border of the liver. A connection is soon made with the hepatic sinusoids of this region and a channel is quickly formed through the liver between the left umbilical and the common hepatic vein (Fig. 5). In an older stage (11.5 mm.) the right vein also sends a branch through the liver to the common hepatic vein.

Thus we see that in turtles as well as in snakes, both umbilicals enter the liver, while in lizards only one, the left, does so. In snakes; however, this secondary connection with the common hepatic vein is made much

earlier; in fact, it appears to occur, according to Hochstetter, before the right vein has been broken up by the hepatic tubules. The postabdominals now open into the iliacs instead of into the postcardinals.

The backward shifting of the point where the umbilical veins enter the liver, and the formation of the first anlage of the left hepatic reventent vein. *Embryo of about 12 mm.*—Both umbilical veins, as we have seen, now send branches through the liver to the common hepatic vein (Fig. 6). The portion of the left umbilical vein in the body-wall craniad of its hepatic branch has completely degenerated. The right vein, on the other hand, still retains for a short time its connection with the sinus

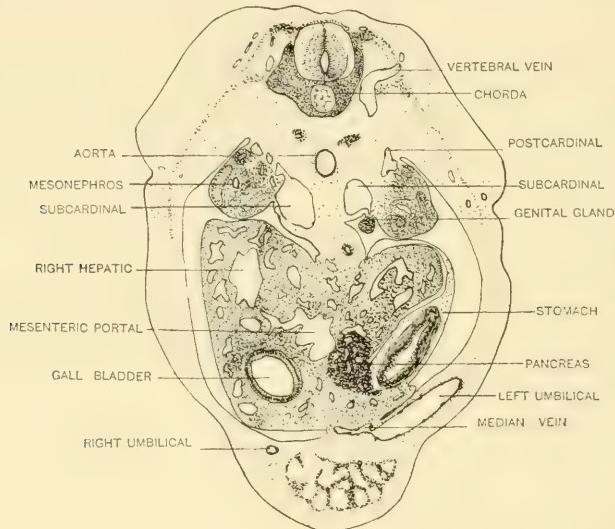


FIG. 8. Cross section of a 13 mm. embryo of *Kinosternon pennsylvanicum*.

venosus, or, more accurately speaking, with the terminal portion of the common hepatic vein (Fig. 6). Before, however, the 12.5 mm. stage is reached there is no longer any direct connection between the umbilical veins and the sinus venosus, so that all of the blood from these veins must pass through the liver in order to reach the heart.

The continued backward growth of the folds of the lateral body-walls that go to form the dorsal pericardium causes more and more of the umbilical vein of each side to lie between the liver and the heart. In the case of the left vein, this results in a shifting backward of the point where this vein enters the liver and a consequent atrophy of the vein craniad of this point. The left umbilical vein does not form an entirely new path through the liver; on the contrary, only that portion marked X

in Fig. 6 is a new formation. The rest is the original path of the 11 mm. stage. The outer portion of the original path (partly hidden in Fig. 6 by the part marked X) is still retained as a tributary of the hepatic umbilical and is later to form a part of the left hepatic revehent vein of the adult.

The umbilical veins join the mesenteric portal vein and lose their connection with the common hepatic. Completion of the anlage of the left hepatic revehent vein. Embryo of 13 mm.—In the 13 mm. stage, the umbilical veins undergo a still further shifting mediad. Just caudad

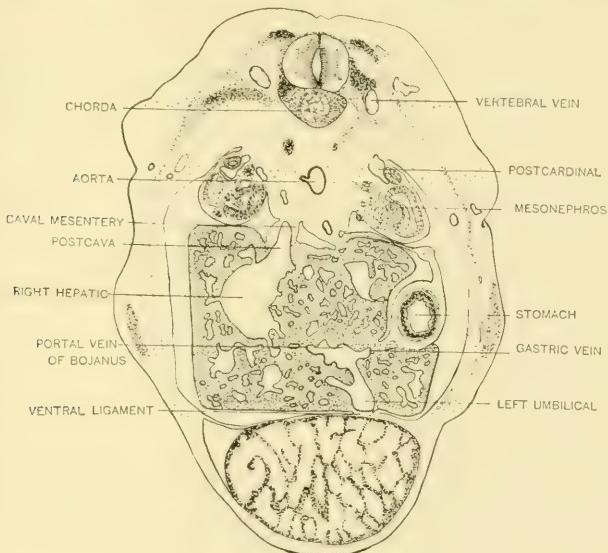


FIG. 9. Cross section of a 13 mm. embryo of *Kinosternon pennsylvanicum* through the level where the postcava enters the liver, showing the union of the left umbilical and gastric vein to form the first anlage of the portal vein of Bojanus.

of the pericardial cavity the right umbilical vein sends a large branch to the mid-line of the ventral body wall, where it receives a small branch from the left umbilical vein (Fig. 8). The median vein formed by the union of these two branches enters the liver through the primary ventral ligament and then immediately divides into two branches. The left branch passes directly dorsad to open into the mesenteric portal, while the right branch soon becomes lost in the hepatic network.

This short median vein of the primary ventral ligament corresponds in position to the "veine mediane" described in birds by Brouha, 98. It does not, however, arise as an independent vessel in the ventral body-

wall, as it does in birds, but is merely the result of the fusion of the two branches of the right and left umbilicals, as shown by Fig. 8. Furthermore, in turtles, it is only a temporary condition and merely marks one stage in the gradual shifting of the position of the umbilical veins.

The right umbilical vein craniad of its connection with the median vein continues forward for a short distance in the body-wall and finally disappears (Fig. 8).

The left umbilical vein continues still further forward and enters the liver a little to the left of the mid-line (Fig. 9). It now forms an entirely new channel through the liver and opens into the gastric vein mentioned in the earlier part of this paper. The gastric vein, as we remember, opened at first into the ventral hepatic anastomosis between the two omphalo-mesenteric veins, then into the right omphalo-mesenteric vein. After the mesenteric portal vein is split off from the right hepatic the gastric vein is split off with it, so that in the present stage it opens into the mesenteric portal vein. That part of the gastric vein which conveys the blood from the left umbilical vein to the mesenteric portal becomes very much larger than the other part and may be considered as the continuation of the umbilical vein. We may, therefore, now say that the left umbilical vein opens directly into the mesenteric portal; and that one of its branches is the gastric vein. The small common trunk formed by the union of the gastric and left umbilical veins is the first anlage of the *portal vein of Bojanus*.

The original path of the left umbilical vein through the liver now becomes a part of the left hepatic vein, the terminal end of which, as we have already seen (Fig. 6, Z), is split off from the right omphalo-mesenteric vein. The left hepatic vein is, therefore, formed from two parts: one, the terminal portion, is split off from the right omphalo-mesenteric vein, while the other portion is the new path formed through the sinusoids of the liver by the left umbilical vein when it first entered that organ.

The ground-plan arrangement of the veins of the liver has now been laid down. The chief events in the further development of the umbilical veins and their derivatives are as follows:

The right umbilical vein makes direct connections with the mesenteric portal, or with one of its branches.

The portal vein of Bojanus lengthens as the liver increases in width.

The common hepatic vein continually grows shorter, being absorbed by the backward extension of the sinus venosus. The result is that the left hepatic vein gradually approaches the sinus venosus and eventually opens into it. A still further caudal extension of the venous sinus

causes an apparent shifting laterad of the left hepatic vein so that this vein finally comes to open into the extreme left end of this organ as in Fig. 1. Some of the small vessels which formerly opened into the left hepatic vein would now open into the sinus venosus, and the number of these would be related to the extent of the backward prolongation of the latter.

The two veins earlier described as the postabdominals become more important as the hinder limbs increase in size. With the degeneration of the allantois they become the chief tributaries of the intra-embryonic umbilicals and with the latter form the abdominal veins of the adult.

The abdominal veins are formed from three anlagen: (1) the hepatic portion is the remains of the last channels formed by the umbilical veins through the liver to the mesenteric portal, or its tributaries; (2) the portion between the liver and the umbilicus is the remains of the original umbilicals which have been carried mediad by the ingrowth of the connective tissue to form the pericardium; and (3) the postabdominals which connect with the iliacs as in the adult.

RENAL PORTAL SYSTEM.

VEINS OF THE ADULT.

Fig. 10 is a schematic diagram of the larger body veins of *Kinosternon pennsylvanicum*.

The afferent veins of the renal portal system of turtles are the anterior renal advehent, the posterior renal advehent, or hypogastric, and the external renal advehent, or iliac veins. The efferent veins from the kidneys, testes (or ovaries) with their ducts, and veins from the suprarenal bodies open into the two root branches of the postcava.

The anterior renal advehent vein is a large ventral branch of the posterior vertebral vein of its side. It comes out between the fifth and sixth ribs and passes over the ventral surface of the kidney to open into the posterior renal advehent vein by an end to end anastomosis. In its course it receives an intercostal vein, and gives off numerous branches to the kidney, testis (or ovary), and suprarenal body. The posterior vertebral veins which supply the blood to the anterior advehents may be briefly considered at this point. This pair of vessels runs the entire length of the body on each side of the spinal column dorsal to the ribs. They receive the first four intercostal veins and veins from the spinal cord which pass out between the vertebrae along with the spinal nerves. Just craniad of the first thoracic vertebra they unite to form a short stem which runs forward a short distance dorsal to the vertebral column,

and then divides into three large branches. One of these branches continues forward just under the skin on the dorsal surface of the neck. It receives a number of branches from the muscles of the neck, and an anastomosing branch from the internal jugular, then divides and enters the skull to open into the occipital sinus. The other two branches pass

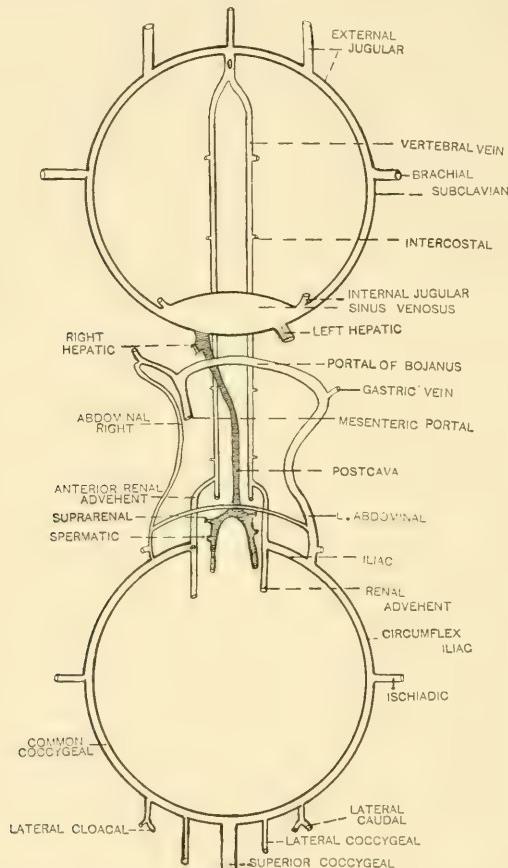


FIG. 10. Diagram showing the larger body veins of *Kinosternon pennsylvanicum*. Ventral view.

around parallel to the anterior border of the shell and open into the external jugular of each side. Caudally they give off branches to the dorsal surface of the kidneys, as well as the large branch to the ventral surface.

The posterior renal advehent veins or the hypogastric veins of Bojanus, 19, arise from a number of small veins from the inner and hinder

parts of the pelvis (especially the external genitals, cloaca, etc.) and pass forward to the ventral surface of the kidneys where they open into the anterior advehent veins in the manner described above. They give off afferent veins to the kidneys, testes (or ovaries) with their ducts, and to the suprarenal bodies. In the large snapping turtle (*Chelydra serpentina*) they also make direct connections with the roots of the postcava. Jourdain, 59, denies that such a direct connection takes place. The writer has not been able to trace such connections in *Kinosternon*, nor, indeed, in all of the snappers. In the smaller turtles these connections could not be made out except by a successful celloidin corrosion even if they were present on account of the extreme smallness of the veins. Bojanus, 19, shows such a connection in his figure 124, plate XXV, O*, of *Testudo europaea*, but this is probably the result of his erroneous idea of the real significance of the veins of the kidney.

The external revehent veins, or the iliacs of Bojanus, arise as dorsal branches of the circumflex iliacs. Each receives, before reaching the kidney, a large branch from external border of the shell (not shown in figure). This border vein receives the terminal ends of the intercostal veins, and the veins from the fat body.

According to Nicholai, 26, and later Martino, 41, all three of these veins act as afferents to the kidneys. Jourdain, 59, supposed, however, that this need not always be the case, but that sometimes the blood current of the iliacs may be reversed. At all events, it appears that the blood from the circumflex iliacs is usually divided so that a part of it must pass through the capillaries of the kidney to the postcava, while another part must pass through the hepatic network before reaching the heart. Therefore, it does not seem improbable that at times a larger amount of blood may pass through the iliacs to the kidneys than at others, and that under certain conditions the blood current in this short vein may actually be reversed.

The circumflex iliac vein (vena iliaca circumflexa) is formed by the union of the ischiadic and the common coccygeal veins. The common coccygeal vein arises from several veins of the caudal region, the more important of which are: the superior coccygeal, the lateral coccygeal, the lateral cloacal, and the lateral caudal. The latter two veins often unite to form a short single stem just before opening into the common coccygeal. The circumflex iliac divides in front of the pelvis into a dorsal and a ventral branch, the iliac and the abdominal veins, respectively.

The abdominal veins extend along the ventral wall of the body from the pelvis to the liver. They are connected in front of the pelvis by a

small anastomosing branch. As has already been stated, they open into the portal veins of the liver.

The postcava arises by two short branches between the kidneys and extends forward as an unpaired vessel ventrolateral to the aorta and, after passing through the liver, opens into the right side of the sinus venosus. The two root branches receive the renal, suprarenal, and spermatic revehent veins. The unpaired portion receives only the revehent veins of the liver.

The Chelonia differ from both the Lacertilia and the Ophidia in the possession of an unpaired postcava.

DEVELOPMENT OF THE RENAL PORTAL SYSTEM.

Development of the postcava.—The veins to be considered in this connection are the postcardinals and the subcardinals. Owing to the fact that these veins are already laid down in the 7.4 mm. embryo, it will not be possible to trace their earliest development in this reptile. According to Rabl, 92, and Hoffmann, 93, who investigated the earlier development of the postcardinals in the selachians, these veins arise by a longitudinal fusion of the intersegmental branches of the aorta. Brouha, 98, in his investigations on the development of the liver in birds, finds that in a chick embryo of 47 hrs., the postcardinals and umbilicals are not yet formed. There is found, however, in the body-walls caudad of the sinus venosus a network of vessels which open into the ducts of Cuvier. In a 62 hr. chick embryo, two vessels, which are still connected by a number of cross branches, are formed from this network. The outer of these vessels is the postcardinal, while the inner represents the umbilical. It is not until the 72 hr. stage is reached that these cross branches completely disappear. Lewis, 94, finds that in the rabbit the postcardinal veins appear later than the umbilicals. "The umbilical vein sends branches toward the aorta, into the posterior limb and less distinctly into the anterior limb. A longitudinal anastomosis of these vessels, uniting with a sprout from the venous end of the heart, produces the posterior cardinal vein, which then is cut off from the umbilical vein, carrying with it the veins from the limbs." (Lewis: Am. Jour. Anat., Vol. III, p. XII.)

In a turtle embryo of 7.4 mm. the postcardinal veins extend forward from the caudal termination of the mesonephric ducts along the entire length of the mesonephroi, dorsolateral to the mesonephric ducts. No distinct caudal vein is yet formed. At the caudal termination of the mesonephric ducts the postcardinal of each side receives a number of small branches from the tail, the last dorsal intersegmental branch, and

a stout anastomosing branch from the subcardinals of the same side. The portion of the postcardinal between the cranial end of the mesonephros and the sinus venosus, that is to say the azygos portion of Lewis, 04, is broken up into two or three vessels; often one large vein and one or two much smaller, all of which open separately into the ducts of Cuvier. While this would seem to correspond with the condition found in lizards, it is interesting to note that in Chelonia the postcardinals retain their identity as distinct vessels throughout their entire course, and are not broken up cranially into broad blood sinuses as they are in lizards. The conditions here met probably represent the remains of the venous plexus described by Brouha in the chick. The postcardinals are still connected with the umbilicals by a number of anastomosing branches.

The postcardinals already receive a series of intersegmental branches from the dorsal body wall and also a number of branches from the mesentery ventral to the aorta. As soon as the anterior limb ridge is formed, it sends six or eight vessels to the postcardinal. These limb veins, as Hoffmann, 90, has already shown in the lizards, comes from between the successive myotomes of which the limb is developed. They also connect at this time with the umbilicals.

The name subcardinals was given by Lewis, 02, to a pair of veins which extend along the ventromedial border of the mesonephroi and correspond to the *venae renales revetentes* of Hochstetter. According to Lewis, 02, 04, the subcardinal veins are split off from the mesonephric portion of the postcardinals ("Mesonephric azygos"). Miller, 03, claims that "The subcardinal veins arise in birds as unconnected vessels or islands, which are without doubt independent structures; and the connections with the postcardinals are formed later and secondarily." (Miller: Am. Jour. Anat., Vol. II, p. 286.)

In a 7.4 mm. turtle embryo the subcardinal veins extend from near the root of the tail forward along the ventromedial border of the mesonephroi to a point somewhat craniad of the origin of the omphalo-mesenteric artery where they open into the postcardinals. The subcardinal veins do not appear to be connected with each other in any way at this stage, although, on the other hand, their connection with the postcardinals is very complete, both through direct connections and through the venous network of the mesonephroi.

Both the postcardinals and the subcardinals are still very small. The mesonephroi are very simple, being scarcely more than a series of segmentally arranged tubules opening into the mesonephric ducts.

As the mesonephroi increase in size and complexity, the postcardinal

and subcardinal veins also become more important. The subcardinal veins unite with the caudal vein as soon as it is formed and they become the advehent veins of the primitive kidneys. Fig. 11 is a reconstruction of the postcardinal and subcardinal veins of a 9 mm. turtle embryo. Only the direct connections between these two sets of veins are shown. The sinusoidal network of the mesonephroi is very complex at this stage. The postcardinals have now reached their maximum of development in their cranial portions, just as have the subcardinals in the caudal region. In other words the primitive portal system of the mesonephroi is now at its height of development.

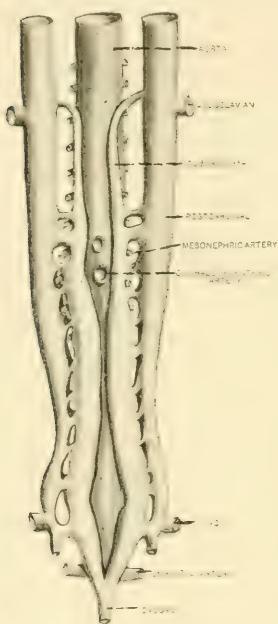


FIG. 11. Frontal reconstruction of the postcardinal and subcardinal veins of a 9 mm. embryo of *Kinosternon peninsylvanicum*. Ventral view.

of the anlage of the postcava. The short vein formed in the caval mesentery seems to grow from the right subcardinal toward the liver, since in the 10 mm. stage, as noted in an earlier part of the paper, it may be traced up to the border of the liver where it apparently breaks up into a number of smaller vessels. It appears to later form a path for itself through the sinusoids of the liver and thus join the right hepatic vein.

In the meantime the two subcardinal veins have become connected by several anastomosing branches which at first are small and of about equal

At about this time a series of changes takes place which ultimately results in the complete reversal of the primitive portal system of the mesonephroi.

These changes are made possible by two important formations: (a) the right hepatic vein, and (b) the caval mesentery. The formation of the right hepatic vein has already been discussed in an earlier part of this paper.

The development of the caval mesentery has been frequently described by a number of investigators, Hoffmann, Hochstetter, and others, so that it is not necessary to take it up at this time. It arises in the cranial region of the body as a bud or process from the median mesentery and grows both ventrally and caudally so that it becomes attached to the dorsal surface of the liver further and further caudad. When it reaches a certain point slightly craniad of the origin of the omphalo-mesenteric artery, a short vein is developed along its caudal border, which unites the right subcardinal with the right hepatic vein and thus completes the formation

of the anlage of the postcava. The short vein formed in the caval mesentery seems to grow from the right subcardinal toward the liver, since in the 10 mm. stage, as noted in an earlier part of the paper, it may be traced up to the border of the liver where it apparently breaks up into a number of smaller vessels. It appears to later form a path for itself through the sinusoids of the liver and thus join the right hepatic vein.

calibre. Later, however, some of these branches increase greatly in size as in Fig. 12 and eventually the two veins fuse throughout almost their entire length caudad of the origin of the omphalo-mesenteric artery to form the unpaired posteava of the adult. In neither *Kinosternon* nor *Chrysemys* do the subcardinals fuse, as a rule at least, in front of the origin of this artery. In only one embryo has the writer seen the slightest indications of such an anastomosis.

The formation of the posteava permits the blood from the caudal regions to enter the heart through the liver; a path which immediately becomes very important since the postcardinals are now beginning to degenerate cranially. Fig. 12 is a reconstruction of the postcardinal and subcardinal veins of a 12 mm. turtle embryo, caudad of the point where the posteava opens into the right hepatic vein. The postcardinals have now lost their connection with the sinus venosus. Caudally, on the other hand, they make strong connections with the caudal vein. The subcardinals are rapidly losing their connection with the caudal; in fact, they are now connected with the postcardinals slightly caudad of the opening of the iliac veins, instead of directly with the caudal. Even this connection is soon lost and the subcardinals (or root branches of the posteava) now only function as revehent veins for the mesonephroi and the portal system is reversed just as it is in lizards and snakes.

As the mesonephroi degenerate from before backward, the subcardinals fuse further and further caudad as mentioned above. When the permanent kidneys are formed in the pelvic region dorsal to the mesonephroi, they also give off veins to the subcardinals. In the oldest stage studied, 25 mm., the subcardinals have fused as far caudad as to the cranial end of the permanent kidneys.

The posteava of turtles is, therefore, formed from the following parts: (a) usually a small part of the common hepatic vein, (b) the right hepatic revehent vein, (c) the hepatic sinusoids dorsal to the right hepatic vein, (d) the vein formed in the caval mesentery, (e) the right

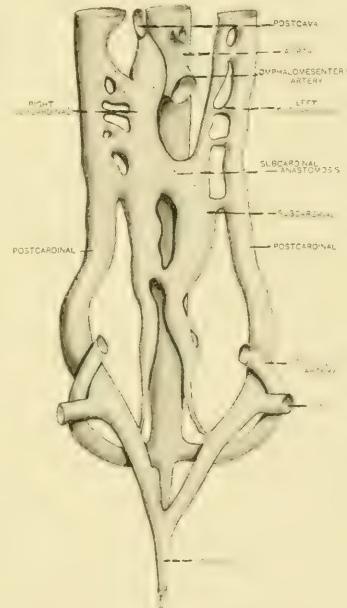


FIG. 12. Frontal reconstruction of the postcardinal and subcardinal veins of a 12 mm. embryo of *Kinosternon pensylvanicum*. Ventral view.

subcardinal craniad of the origin of the omphalo-mesenteric artery, and (f) the fused subcardinals caudad of this point.

The *posterior vertebral veins* are formed in exactly the same manner as in lizards except that the longitudinal fusion of the dorsal intersegmental branches of the postcardinals takes place dorsal to the rib anlagen instead of ventral to them. These veins persist in the adult form.

The *anterior and posterior advehent veins of the permanent kidneys* represent the remains of the postcardinals. The connection between the anterior renal advehent vein and posterior vertebral vein is formed through the persistence of one of the dorsal branches of the primitive postcardinal.

RÉSUMÉ.

In Chelonia the unpaired mesenteric portal vein is formed, in the abdominal region, as in other reptiles, from the originally paired omphalo-mesenteries. Within the liver it is split off from the right omphalo-mesenteric vein.

The cranial termination of the left hepatic revehent vein as well as the greater part of the right revehent of the liver is also split off from the right omphalo-mesenteric.

Both umbilical veins enter the liver and at first open into the common hepatic vein; later they join the portals.

During development the umbilical veins are carried mediad by the ingrowth of the connective tissue folds which are to form the pericardium; and also enter the liver further and further caudad. Eventually they come to form the pre-umbilical portion of the abdominals. The portion of the abdominals behind the umbilicus is developed from a pair of veins which extend forward along the ventral abdominal walls from the iliac veins and open into the umbilicals at the umbilicus.

The portal vein of Bojanus is not developed from the omphalo-mesenteries, but from a venous trunk formed by the union of the left umbilical with the gastric vein.

The postcava is formed from the following parts: (a) the common hepatic vein, (b) the right hepatic revehent vein, (c) sinusoids of the liver, (d) a new venous formation in the caval mesentery, (e) the right subcardinal craniad of the origin of the omphalo-mesenteric artery, and (f) the fused subcardinals caudad of this point.

The vertebral veins develop as a longitudinal fusion of the dorsal intersegmental branches of the postcardinals above the costal anlagen.

The caudal portions of the postcardinals are retained, as in other reptiles, as the anterior and posterior advehent veins of the permanent kidneys.

In general the development of the veins of the hepatic and renal portal systems is the same in turtles as in lizards and snakes. The more important differences are noted below:

Turtles differ from lizards as follows:

1. The left umbilical vein loses its connection with the sinus venosus before the posterior venous ring around the intestine is formed.
2. Both umbilical veins enter the liver, and both persist, in part, to form the pre-umbilical portion of the abdominals of the adult.
3. The subcardinals do not form a venous ring around the origin of the omphalo-mesenteric artery; hence, the right subcardinal, instead of an unpaired stem in front of the origin of this artery, joins the right hepatic vein to form the postcava.
4. The postcava is a single vessel, in the adult, back as far as the cranial ends of the permanent kidneys, where it bifurcates into two branches which receive the suprarenal, renal, and spermatic veins.
5. The posterior vertebral veins are developed dorsal to the anlages of the ribs.
6. The postcardinals are not broken up in the early stages into broad blood sinuses by the mesonephric tubules as they are in lizards, but retain their integrity as independent vessels along their entire length.

Turtles differ from snakes as follows:

1. The right emphalo-mesenteric vein is, at first, smaller than the left.
2. The right umbilical vein does not enter the liver until after the left has done so.
3. Both umbilicals eventually open into the portals.
4. The postcava does not open into the common hepatic vein formed by the union of the right umbilical with the right hepatic, but into the right hepatic before the latter joins the common hepatic.
5. The postcava, as stated above, is unpaired.
6. The postcardinals are not, at first, connected with the caudal, but a connection is later and secondarily formed.
7. The posterior vertebral veins are formed above the rib anlages, and persist in the adult.

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PROCEEDINGS OF THE ASSOCIATION OF AMERICAN ANATOMISTS

EIGHTEENTH SESSION.

*Wistar Institute of Anatomy, Philadelphia, Pennsylvania,
December 27, 28 and 29, 1904.*

At its business sessions the Association took the following actions:

The secretary's minutes were corrected to the extent of making the following order, Section 2 of Article VII of the constitution, as was the intention of this Association by the action taken at its Washington meeting (1902): "Any change in the constitution of this Association must be presented in writing at one meeting in order to receive consideration and be acted upon at the next meeting; due notice of the proposed change to be sent to each member at least one month in advance of the meeting at which such action is to be taken."

Section 2 of Article VII of the constitution as prior to this change thus becomes Section 3 of Article VII.

The Committee of Coöperative Investigation reported as follows:

"Circulars were sent to all members of the Association in September, 1903, requesting that observations be made upon three topics, namely:

"1. The origin, insertion and nerve supply of the brachialis anticus.

"2. Variations in the hepatic artery.

"3. Variations in the external origin of the spinal accessory nerve.

"The circulars also contained a request that the data collected upon any or all of these topics should be reported to the chairman of this committee and a similar request was recently included in a notice issued by the Secretary of the Association.

"The committee regrets to state that but three reports have been received, too few to be of value in the line of investigation which the committee had in view. It would seem that for various reasons the members of the Association are not interested in the object for which the committee was appointed and the committee therefore requests that it be discharged."

Respectfully submitted,

J. PLAYFAIR McMURRICH, *Chairman.*

AMERICAN JOURNAL OF ANATOMY.—VOL. IV.

II Proceedings of the Association of American Anatomists

On motion this report was accepted and the committee discharged with thanks.

It was moved by Doctor Burt G. Wilder and seconded by Doctor Edw. Anthony Spitzka:

1. That there be appointed a Committee on Brain Bequests and Methods.

2. That said committee be requested to report at as early a meeting as practicable upon the following points: (a) A General Form of Brain Bequest; (b) Methods of removing the brain and of its transportation, preservation, study, delineation and description.

3. That the committee consist of Doctors Ales Hrdlicka, Edw. Anthony Spitzka and Burt G. Wilder.

Motion was carried and Doctor Burt G. Wilder was made chairman of the committee.

Forty-one new members were elected.

Dr. Charles R. Bardeen was elected member of the Executive Committee for term expiring in 1909.

TREASURER'S REPORT FOR THE YEAR 1904.

Balance on hand December 24, 1903.....	\$126.94
Dues for 1904.....	800.00
	<hr/>
	\$926.94 \$926.94

Expenditures for 1904:

Expenses of Secretary for Philadelphia meeting, 1903...	\$24.00
To Association's share of expenses for last Congress of American Physicians and Surgeons.....	84.80
To American Journal of Anatomy for 162 subscriptions at \$4.50.....	729.00
To American Journal of Anatomy for reprints of Proceedings and Abstracts of papers.....	8.85
Envelopes and postage.....	18.00
Printing	14.25
	<hr/>
	\$878.90 \$878.90

Balance on hand December 24, 1904..... \$48.04

Dr. Charles S. Minot, Embryological Laboratory, Harvard Medical School, Presidential Address.—Genetic Interpretations in the Domain of Anatomy. (*See American Journal of Anatomy, Vol. IV, No. 2.*)

ABSTRACTS OF PAPERS PRESENTED.

SOME CURRENT TERMINOLOGIC INCONSISTENCIES THAT ARE APPARENTLY NEEDLESS. By BURT G. WILDER. *Cornell University, Ithaca, New York.*

A NEW FORM OF BRAIN BEQUEST. By BURT G. WILDER. *Cornell University, Ithaca, New York.*

REPORT OF A STUDY OF THE BRAINS OF SIX EMINENT SCIENTISTS AND SCHOLARS BELONGING TO THE AMERICAN ANTHROPOMETRIC SOCIETY; TOGETHER WITH A BRIEF DESCRIPTION OF THE SKULL OF ONE OF THEM. (Specimens, drawings and charts.) By EDW. ANTHONY SPITZKA. *Department of Anatomy, Columbia University, New York City.*

A report was made on the brains of Prof. Joseph Leidy, Dr. Philip Leidy, Dr. A. J. Parker, Prof. Harrison Allen, Prof. E. D. Cope and Dr. William Pepper, together with a brief description of the skull of Prof. Cope. The specimens belong to the collection of the American Anthropometric Society, an association of scholars and scientists having for its object the preservation and study of the brains and other bodily organs of its members, which was founded in 1889 or 1890 by Drs. Joseph Leidy, Harrison Allen, William Pepper, F. X. Dercum and E. C. Spitzka. The study of the specimens extended over a period of two years, being conducted in connection with similar researches on many other human brains of various classes and races of men. Systems of brain measurement were devised and applied to all the brains to aid in the comparative study. The entire work, fully illustrated, will be published from the Wistar Institute, where the brains of the six men referred to are now deposited.

The brain-weights are:

Philip Leidy	1415	grams
E. D. Cope	1545	"
Harrison Allen	1531	"
Wm. Pepper	1593	"

The weight of Prof. Leidy's brain may be estimated at 1545 grams at least; it may have weighed more. Dr. Parker's brain probably weighed about 1415 grams. The main morphological characteristics and contrasts among the brains were briefly pointed out, the brains of the two Doctors Seguin, Maj. J. W. Powell, George Francis Train and Maj. J. B. Pond being included in the comparisons. Among the many interesting results of the study, a comparative tabulation of the cerebro-cerebellar ratio of weight showed this to be fully a unit higher than among ordinary men

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(1:7.5). The cross-section area of the callosum among 10 eminent men, ranging from 5.7 to 10.6 sq. cm. (Joseph Leidy) averaged 7.3 sq. cm.; among the same number of ordinary men, the range was 4.7 to 6.7 sq. cm., averaging 5.6 sq. cm. The significance of this redundancy in its relations to the greater elaboration of brain structure as expressed by this commissural system was discussed in detail but cannot be abstracted here.

The skull of Prof. E. D. Cope is in fairly good condition and is remarkable for the proportionately large size of the cranium as compared with that of the face, in this respect approaching that notable skull of Immanuel Kant. The parietal bones are notable for their large expanse. The cranial capacity is 1645 cubic centimeters. Full measurements and stereographic drawings have been recorded.

ON A RACIAL PECULIARITY IN THE BRAIN OF THE NEGRO. By ROBERT BENNETT BEAN. *Department of Anatomy, Johns Hopkins University.*

In a study of thirty-seven brains of the American Negro, and after careful measurement and comparison (individually and collectively) with seventeen brains removed from American Caucasians, it is found that the anterior part of the Negro brain is smaller than the anterior part of the Caucasian brain. The difference varies up to ten millimeters actual linear measurement over the anterior association area in brains of the same antero-posterior diameter. This is apparently more marked in women than in men. The uniformity of the difference is striking when we consider that the American Negro contains various strains of Caucasian blood.

We believe we have found that the anterior association area with its connections is smaller in the American Negro than in the American Caucasian. The posterior part of the Negro brain may be relatively larger than the posterior part of the Caucasian brain, but this is not so marked, nor is it constant. We present only a preliminary note and hope to make a complete report after the examination of a larger number of brains.

THE MAMMALIAN LOWER JAW. By J. S. KINGSLEY. *Tufts College.*

In several works, notably the manuals of human anatomy, the statement occurs that the lower jaw in the mammals ossifies from several centers, but, except in a brief paragraph in one of Kitchen Parker's publications, I have found no attempt to homologise these elements with the bones occurring in the mandible of the lower vertebrates. The work described has been carried on by means of wax reconstructions of the region concerned in embryos of the pig.

In the lower jaw are two cartilaginous elements, the well-known Meckel's cartilage and a second very large cartilage lying outside the posterior part of the Meckelian and extending downward to form the angle of the definitive jaw and upwards to form the condyle for articulation with the glenoid fossa of the squamosal. This condyloid cartilage, which ossifies by endochondrostosis, the author, like Parker, would homologise with the lower labial cartilage of the elasmobranchs which can be traced upwards at least as far as the ganoids, where it occurs in Polypterus (Van Wijhe) and Amia (Allis). In front this condyloid bone becomes confluent with the dentale, a membrane bone arising on the external surface of the Meckelian cartilage. On its anterior-superior margin is a second membrane bone, the coronoid, which forms the coronoid process of the upper jaw. On the inner side of the dentale occurs a third membrane bone in the position of the splenial of the lower vertebrates, while the inner alveolar margin of the anterior end of the dentale may prove to be the prosplenial, but this point has not yet been decided. The articulare is, as has been pointed out in a previous paper on the ossicula auditus, to be found in a part of the malleus, to which a distinct membrane bone, the angulare, also contributes.

NOTES ON THE ORIGIN OF THE CAROTID GLAND AND THE MORPHOLOGICAL COMPARISON OF THE TRIGEMINAL AND FACIAL NERVES IN MAMMALIAN EMBRYOS. By HENRY FOX. *The Temple College, Philadelphia.*

According to one view, the carotid gland arises by a thickening of the walls of the capillaries derived from the carotid artery; according to another view, which receives its strongest support in the careful investigation of Prenant, the organ arises from the endoderm of the third pharyngeal pouch. The author's investigations support the latter view. In a pig embryo of 9 mm., the gland appears as a series of blind follicular outgrowths from the anterior wall of the third pharyngeal pouch. By the study of several succeeding stages, these outgrowths were traced into the definitive carotid gland of later stages. Similarities in structure in the case of the trigeminal and facial nerves are strikingly brought out in the study of early pig embryos. The trigeminal nerve bears the same relation to the mouth (stomatodeum) that the facial does to the first pharyngeal pouch. The Gasserian ganglion lies above the angle of the mouth; the geniculate, above the first pharyngeal pouch. Each ganglion gives off two nerves, one of which passes in front of the corresponding pouch, the other behind it. The former corresponds to the pretrematicus of lower vertebrates; in the mammals it becomes the superior maxillary nerve in the case of the trigeminal; in the case of

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the facial it becomes the large superficial petrosal nerve. Both nerves, superior maxillary and petrosal, arise exclusively from the ganglia. The posterior branch on the other hand, is joined by fibers from the ventral roots of the nerves and hence in both cases the branch thus formed—the postrematicus—is a mixed nerve. In the case of the trigeminal, it becomes the inferior maxillary nerve; in the case of the facial it forms the main trunk of the nerve. The latter divides in the hyoid arch. The anterior branch which receives most if not all of the fibers derived from the ganglion passes beneath the point of union of the first pharyngeal pouch with the skin and enters the mandibular arch; it becomes the chorda tympani nerve. The posterior branch remains in the hyoid arch, its fibers being distributed to the muscle anlagen; it corresponds to the facial proper of the adult.

The ophthalmic branch of the trigeminal has no counterpart in the facial nerve in the mammalia.

NOTE ON THE LIGAMENTS OF THE MAMMARY GLAND. By WILLIAM KEILLER. *Medical Department, University of Texas, Galveston.*

Besides the cutaneous ligaments of Cooper, the fibrous tissue of the mammary gland spreads out peripherally in all directions so as to form a continuous sheet, which binds the gland to the deep fascia beneath the clavicle, along the side of the sternum, to the fascia of the lower costal region and to the fascia of the floor of the axilla. This fascia forms the true suspensory ligament of the gland. It is rich in lymphatics which run in all directions and is of the utmost surgical importance. So far as the writer has been able to inquire, it is not adequately described.

A NOTE ON THE DEVELOPMENT OF THE OESOPHAGEAL EPITHELIUM. By R. H. WHITEHEAD. *Department of Anatomy, University of North Carolina.*

Within the past decade a number of observers have reported finding in the upper part of the human oesophagus small areas which greatly resemble the mucous membrane of the cardiac end of the stomach containing tubular glands similar to the cardiac and fundus glands. In the attempt to explain their occurrence, some authors maintain that they are due to developmental disturbances, that they result from a downgrowth of ectodermal epithelium from the oral cavity, which replaces the original endodermal epithelium over the entire oesophagus, except in minute areas which remain and develop like the gastric epithelium. Others hold that these areas are normal structures and two authors have reported finding their anlagen in human embryos. The writer finds that in the pig the stratified squamous epithelium of the oesophagus is developed *in loco* from

the original epithelium of the foregut, and that there is no downgrowth of ectodermal epithelium from the oral cavity. This conclusion is in accord with the observations of Neumann and of Shaffer of human embryos. In the pig, however, the details of the process are somewhat different, chiefly in the absence of ciliated cells at any time; nor were any appearances noted which might be interpreted as anlagen of the superficial tubular glands mentioned above.

METHODS OF PREPARING THE SALIVARY GLANDS FOR STUDY AND MUSEUM PURPOSES. By CHARLES F. SYLVESTER. *Department of Biology, Princeton University.*

MORPHOLOGY OF THE SALIVARY GLANDS. (From Material at Columbia and Princeton Universities.) By CHURCHILL CARMALT. *Department of Anatomy, Columbia University, New York City.*

With lantern slides from photographs and drawings of specimens in the Museum of Columbia University and Princeton, was presented the salivary apparatus of Alligator mississippiensis, Chelonia mydas, Ornithorhynchus anatinus, Echidna hystricula, Didelphys virginiana, Petauroides volans, Tamandua bivittata, Myrmecophaga jubata, Dasypus sexcinctus, Lepus sylvestris, Sciurus carolinensis, Manatus americanus, Equus caballus, Bos taurus, Canis familiaris, Arctocephalus, Scalops aquaticus, Pteropus, Cynocephalus, Macacus inuus and human parotid, submaxillary, sublingual, labial, pharyngeal, anterior and posterior lingual, palatal and uvular glands. Cross sections, models and corrossions of these same human glands illustrate relations.

In conclusion there is morphologic constancy and identity of salivary apparatus throughout the mammalian series.

There is a group of glands along the alveo-buccal borders with compound acinous elements, the parotid at its posterior or caudal union.

There is a group of glands along the alveo-lingual groove, the anterior end of which becomes compound with one, two or three ducts in different orders, represented as variations in human subjects.

The glands correspond in size with the digestive function, herbivorous animals having large, carnivorous small glands. This only applies in a wider sense, for carnivores like the bear with herbovore habits grow larger glands.

The submaxillary gland is dependent for its size upon mobility of the tongue.

Salivary glands increase in size with age and with pregnancy.

TOPOGRAPHY OF THE PANCREAS IN THE HUMAN FOETUS. By C. M. JACKSON. *Department of Anatomy, University of Missouri.*

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PRELIMINARY REPORT OF EXPERIMENTAL WORK AND OBSERVATIONS ON THE AREAS OF LANGERHANS IN CERTAIN MAMMALS. By LYDIA M. DE WITT. *Department of Histology and Embryology, University of Michigan.*

I. By wax-plate reconstruction of injected and uninjected areas of Langerhans in man, rabbit, cat and rat, it is seen that these islands are spherical or oval or irregular or lobulated in form, vary greatly in size, both in animals of the same species and in animals of different species; they consist of anastomosing irregular cords of cells forming a sponge-like structure. One or several thin-walled blood vessels pass in tortuous course through the central part of each area, divide several times, the branches being connected by many anastomosing capillaries. Numerous capillaries and several larger vessels leave the areas and communicate freely with the interacinar blood vessels. The large and irregular size of the vessels, the thin walls and the similarity in structure of the afferent and efferent vessels indicate that the circulation in the areas is sinusoidal.

II. The operations on the twenty-eight cats used consisted in ligation of the duct, division of the gland between two ligatures, cauterization of the cut ends and sometimes tying a fold of omentum around the cut ends of the gland. The animals were killed in from three to two hundred days after the operation. Portions of the gland were removed for microscopic study and the remainder extracted with water or glycerine and its digestive action on proteids, starches and fats and also its glycolytic action tested, the results in all cases being controlled by similar tests with extracts of the normal portion of the same pancreas. The microscopic changes consisted of atrophy of lobules and acini and increase of connective tissue, the areas of Langerhans being in most cases well preserved. In seven of the most successful experiments, the amylolytic, steatolytic and proteolytic action was absent and in many others it was much diminished, while the glycolytic action was not in any case diminished.

My results seem therefore to point to the conclusion that the areas of Langerhans are vascular glands having a sinusoidal circulation in intimate relation with the acinal blood supply; that they have a secretion whose glycolytic action is equal to that of the normal pancreas and which corresponds in its reactions and behavior to the activator principle of the pancreas described by Cohnheim.

ON THE ANATOMY OF A 4.0 MM. EMBRYO FROM THE HARVARD EMBRYOLOGICAL COLLECTION. By J. L. BREMER. *Embryological Laboratory, Harvard Medical School.*

THE TOTAL FOLDS OF THE FOREBRAIN, THEIR ORIGIN AND DEVELOPMENT TO THE THIRD WEEK IN THE HUMAN EMBRYO.
By SUSANNA PHELPS GAGE. *Laboratory of Histology and Embryology, Cornell University.*

The first pair of total folds appears in birds and lower forms when the flat neural plate is molded over the blind end of the enteron. With the growth of the neural plate and its dipping downward over the end of the enteron, these folds are carried with it. This early association of enteron and neural plate is soon lost, the notochord first and the mesoderm later intervening between the end of the enteron and the part of the neural plate just mentioned. These first neural folds later become the albicantial folds and in mammals (man and cat) are relatively most conspicuous when the gill clefts are at their height of development (third to fifth week in man). In man while the neural plate is growing forward, but is still widely open (*Homo 12*, Johns Hopkins University Collection), these albicantial folds and those giving the first indication of eyes can be seen. As the plates further coalesce (*Homo 714*, Harvard University embryological collection), in addition to the above folds and nearer the margin of the plate, are two pairs of folds representing the olfactory and diencephalic regions. With the final closure of the plate to form the neural tube (*Homo 209*, Johns Hopkins University collection), the four pairs of folds already formed, albicantial, visual, olfactory and diencephalic, unite to form corresponding lobes.

At three weeks, there being still a remnant of the neuropore which marks the point of the closure of the neural tube (*Homo 148*, Johns Hopkins University collection), these four lobes show further differentiation: (a) the albicantial being divided into (1) the albicantial and (2) hypophyseal folds; (b) the visual being divided into (3) the eye vesicle and (4) the eye stalk; (c) the olfactory divided into (5) the striatal, (6) the olfactory and (7) the cerebral folds; (d) the diencephalic divided into two folds (8 and 9). That is, at the end of three weeks the main folds seen in the forebrain are already outlined.

The human embryos above mentioned were placed at my disposal by Drs. Mall and Minot for comparison with the large series of mammalian and immammalian material in the embryological collection of Cornell University.

THE DEVELOPMENT OF THE PARAPHRYSIS AND THE PINEAL REGIONS OF NECTURUS. By JOHN WARREN. *Embryological Laboratory, Harvard Medical School.*

The paraphysis appears first in an embryo of 12 mm. as a small diverticulum from the roof of the forebrain anterior to the velum

transversum. The epiphysis arises from the roof of the midbrain some little distance posterior to the velum at a slightly earlier stage. At 15 mm. the paraphysis is a slender tube parallel to the velum and opening just anterior to its lower extremity. The roof of the forebrain just anterior to it has grown downward to a marked degree so that a very marked angle has been formed in the roof of this part of the brain. The epiphysis is attached to the brain by a short stalk which, however, contains no cavity and the posterior commissure appears for the first time just behind the stalk. From now on the paraphysis begins to give off lateral tubules. It lies in close relation to two vessels, one anterior and one posterior. The velum grows rapidly backward nearly to the hindbrain and another outgrowth arises from the roof of the forebrain just anterior to the paraphysis and extends downward and backward towards the infundibular recess. The vessels in relation to the paraphysis grow into these projections and form two choroid plexuses, a superior and an inferior, between the origins of which opens the paraphysis. The choroid plexus of the lateral ventricles springs from the base of the inferior plexus. The superior commissure appears first at 16.5 mm. In the adult the paraphysis extends forward between and above the hemispheres. From its central cavity, a confused mass of tubules are given off, which appear to anastomose to a certain extent and each tubule is in intimate relation with a vessel, no connective tissue lying between them. This is apparently a type of sinusoidal circulation and the structure is of a glandular character. The choroid plexuses are very extensive and surround the opening of the paraphysis. They intermingle to a great extent with each other and extend backward to the hindbrain. The epiphysis is a narrow tube extending forward over the superior commissure and connected to the brain by a short slender stalk. Its cavity is subdivided to a certain extent by incomplete septa.

THE DEVELOPMENT OF THE CUTANEOUS NERVES OF THE POSTERIOR LIMB IN MAN. By CHARLES R. BARDEEN. *Department of Anatomy, University of Wisconsin.*

The cutaneous nerves of the posterior limb in man first extend out to the anterior, distal and posterior margin of the limb-bud and from these margins they then grow over the ventral and dorsal surfaces of the limb. The segmental distribution of the sensory fibers of the spinal nerves which supply the limb described by Head, Sherrington and others, seems to be due to the fact that each spinal nerve has a path of maximum directness toward the margins of the limb bud at the period when the sensory branches of the main nerves of the limb are given off.

NORMAL PLATES OF THE DEVELOPMENT OF THE RABBIT. By
EWING TAYLOR. *Embryological Laboratory, Harvard Medical School.*

The standard plates of the development of the rabbit will form one of the series of "Normentafeln zur Entwicklungsgeschichte der Wirbeltiere" edited by Prof. Franz Keibel of Freiburg in Breisgau. The work will have three plates. Two of these will show, on a uniform magnification of five diameters, the entire series of embryos, extending from the stage of the blastodermic vesicle with a circular embryonic shield up to the twenty day condition. The third plate will show the young stages up to and including the ten and a half day embryo on a magnification of twenty or ten diameters. A description of the embryos corresponding to the figures on the plates, will constitute the text of the Normentafeln. A tabulation of the internal development of the rabbit, based on a study of the serial sections, will be included in the work. Three series, cut in three different planes, but measuring approximately the same, were studied for each period of development, except in the younger stages of eight and a half days and under. The specimens for each period were obtained, except as otherwise noted, by selection from several litters of the same age. The entire work is practically completed, excepting the bibliography, which now contains more than five hundred titles.

THE ANATOMY OF THE INGUINAL AND FEMORAL REGIONS WITH SPECIAL REFERENCE TO THE ARRANGEMENT OF THE FASCIA.
By ARTHUR S. VOSBURGH. *Department of Anatomy, Columbia University, New York City.*

The subject was presented from two standpoints:

1st. A description of the structures, layer by layer, illustrated by diagrams, charts and drawings.

2nd. The same structures were shown in a series of sagittal and transverse sections through the groin and proximal portion of the thigh.

(Sections and Drawings.)

The following points were emphasized:

1. Formation of triangular ligament from fibers derived from medial pillar of opposite side.

2. Absence of anything that can consistently be described as "Conjoined tendon." The structure usually so described is a small dorsal lamina of the internal oblique split off from the main muscular mass by the passage of the abdominal nerves. The structure often mistaken for "conjoined tendon" is the transversalis fascia forming the dorsal

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wall of the inguinal canal, here strengthened by scattered fibers derived from the internal oblique and the transversalis.

3. The impossibility of "conjoined tendon" forming a covering for "direct inguinal hernia," it being a thin or thicker portion of transversalis fascia according to point of impact.

4. Formation and description of the fascial compartment in which the femoral vessels lie. The femoral sheath was described as made up solely of areolar tissue. This areolar tissue is continuous with the areolar sheaths of vessels and with the pro- and retro-peritoneal tissue.

5. Femoral canal does not exist. It is simply a complementary space mesad to the vein, filled with lymphatic structures and areolar tissue.

GLYCOGEN IN ANIMAL TISSUE. By SIMON H. GAGE. *Laboratory of Histology and Embryology, Cornell University.*

Assuming the correctness of Bernard's generalization, that in the course of development the liver gradually assumes and the rest of the tissues and organs of the body lose the glycogenic function, it was hoped that the presence or absence of glycogen in the blind sac, the so-called liver, of *Amphioxus* might aid in determining its homology with the liver of higher vertebrates. Abundant material was obtained directly from the ocean at the Bermuda Biological Station during the session of 1904.

For comparison the most closely related fresh-water form, the *Ammocetes*, obtained from Cayuga lake inlet, was used.

In *Amphioxus* and the related form, *Asymmetron*, the blind sac was found not to possess a specialized glycogenic function; but glycogen was found in the intestinal and branchial epithelium, also in the epidermis and the nerve cells of the central nervous system.

In *Ammocetes*, glycogen was found in the liver, the intestinal epithelium, the epidermis, the heart and skeletal muscles, in the central nervous system, cartilage, the notochord and in fat cells.

In the horse it was found that the glycogenic function was not limited in the adult to the liver, but persists in the skeletal and cardiac muscles.

The main results gained are then: (a) In some animals at least, the glycogenic function of the adult (and the independently feeding lamprey or *Ammocetes*) is not restricted to the liver, but persists as a function of the tissues generally.

(b) Glycogen is apparently present in the *Amphioxus* and *Asymmetron* only for a short time after feeding. These animals and *Ammocetes* are constant feeders and with both a brief fast is sufficient to free them from glycogen.

The most important result was the discovery of abundant glycogen in the nerve cells of the myel (spinal cord) of Amphioxus and Asymmetron, and also in the brain of Ammocœtes. All previous observers have stated that glycogen is absent from the nervous system of vertebrates.

KARYOKINETIC DIVISION IN THE SPINAL GANGLION CELLS OF TRITON LARVÆ. By Ross G. HARRISON. *The Anatomical Laboratory, Johns Hopkins University.*

In Triton larvae (10-13.5 mm. in length) spinal ganglion cells with long and defined process were found undergoing karyokinetic division.

ON THE HISTOGENESIS OF SPINAL GANGLIA IN MAMMALS. By GEORGE L. STREETER. *Department of Anatomy, Johns Hopkins University.*

Spinal ganglia of the chick, pig and man were studied by comparing teased preparations with preserved material cut in paraffin. The observations are as follows:

1. The cells of the neural crest, as they separate off and migrate ventralward between the myotome and neural tube, possess elongated vacuolated cell bodies and appear in well preserved material to fuse into cell clumps, possibly a syncytium. At this time ganglion cells cannot be distinguished from capsule and sheath cells.
2. Neural crest cells are differentiated into—
 - a. *Ganglion cells.*—Formation of processes. Condensation of protoplasm of cell body. Appearance of basic stainable substance on periphery. Increase and clumping of stainable substance (Nissl bodies). Nucleus eccentric and comparatively late it retires to center of cell and assumes resting appearance.
 - b. *Capsule cells.*—Cell body converted into branching flat irregular processes which envelop early the ganglion cells and resemble neuroglia cells. Capsule cells are identical with sheath cells.
3. The early ganglion cells have a fusiform shape with processes at the poles. Between these are found cells which develop later as a secondary growth and are characterized by greater irregularity in shape of cell body, and in situation, size, and number of processes.
4. Many bipolar cells in pig and man do not undergo a transformation into the Ranvier T cells. Some unipolar cells seem to be unipolar from the first.
5. Multipolar ganglion cells are found in considerable variety in adult human spinal ganglia.

MARKED DIFFERENCES BETWEEN THE SKIN OF THE MALE AND THAT OF THE FEMALE FROG. By A. O. FISHER. *Department of Anatomy, University of Wisconsin.*

During the fall the dermis of the female frog is thinner and is less resistant to acids and alkalies and digestive fluids than that of the male. These changes seem to be associated with the formation of the ova.

THE BLOOD AND LYMPH VESSELS OF THE LUNG OF NECTURUS. By WILLIAM S. MILLER. *Department of Anatomy, University of Wisconsin.*

THE MESENTERY IN AMPHIBIA AND REPTILIA. By WILLIAM S. MILLER. *Department of Anatomy, University of Wisconsin.*

In this note, attention was called to the lesser peritoneal cavity (*bursa hepato-enterica*) in *Necturus*, *Cryptobranchus*, *Ambystoma* and *Chrysemys*.

In *Necturus* the ligamentum hepatogastricum is attached to the liver along the course of the hepatic-portal vein and to the cephalic half of the stomach, the gastric attachment being less than the hepatic. Between the caudal margin of this ligament and the entrance of the ductus choledochus into the intestine there is complete absence of any mesentery on the ventral side of the stomach. The ligamentum hepato-cavo-pulmonale extends as an unbroken fold from the liver to the mid-dorsal body wall. The dorsal mesentery caudal to the splenic blood vessels has a large perforation. There are therefore two openings into the *bursa hepato-enterica*, one ventral to the stomach, the other at the caudal end of the dorsal mesentery. These two openings are already present in embryos 25 mm. in length.

In *Cryptobranchus* the intestine forms a long loop after leaving the stomach. The liver is connected with the stomach and intestinal loop by a ligamentum hepato-gastro-duodenale and it is without any perforation. A ligamentum hepato-cavo-pulmonale is present and it also is intact: but caudal to the ductus choledochus there is an opening leading into the *bursa hepato-enterica*, a true foramen epiploicum. The dorsal mesogastrium has a wide opening in it caudal to the splenic blood vessels and in this respect shows a less complete bursa than *Necturus*.

In *Ambystoma* the ligamentum hepatogastricum extends the entire length of the liver, but like *Necturus*, its gastric attachment is shorter than its hepatic; the opening between its caudal margin and the ductus choledochus is smaller than in *Necturus*. The ligamentum hepato-cavo-pulmonale is without any perforation. The dorsal mesogastrium has undergone a very considerable modification. Only the extreme cephalic end of the stomach is in connection with this membrane; it is then

continued along the mesial border of the anterior half of the left lung where it ends. A second remnant of the dorsal mesogastrium is found in the ligamentum gastro-lienale connecting the stomach and spleen. The bursa hepato-enterica is therefore much less complete in *Amblystoma* than in either of the two preceding forms.

In *Chrysemys*, the bursa hepato-enterica, like that of mammals, is connected with the general body cavity in the earlier stages of development; but as development goes on, the cavity becomes much enlarged, takes an irregular H shape and is finally cut off from all communication with the general body cavity. The spleen is situated in its own pocket which may or may not communicate with the bursa hepato-enterica.

ON THE SHAPE OF THE URINIFEROUS TUBULES OF MAMMALS. By G. CARL HUBER. *Department of Histology and Embryology, University of Michigan.*

American Journal of Anatomy, Vol. IV. (*Supplementary number.*)

CYTOTOLOGICAL CHANGES IN THE KIDNEY DUE TO DISTILLED WATER AND VARYING STRENGTHS OF SALT SOLUTION. By FERDINAND SCHMITTER. *Department of Anatomy, University of Wisconsin.*

Fresh kidney tissue macerated in distilled water shows large vesicles within the lumen such as are usually seen in chronic interstitial nephritis. Bütschli's schaumstruktur appears in the epithelial cytoplasm. Imbrication of epithelium occurs in places. Bowman's capsule becomes greatly distended. Maceration in hypertonic salt solution causes appearance of brush border in convoluted tubules. Strong solutions also cause vacuoles and canals to appear within the cytoplasm.

RECENT ADDITIONS TO THE HARVARD EMBRYOLOGICAL COLLECTION. By CHARLES S. MINOT. *Harvard Medical School.*

DEMONSTRATIONS.

1. Dr. Charles R. Bardeen: Models of *a*, The development of the thoracic vertebræ in the human embryo; *b*, The development of the skeleton of the leg in the human embryo.
2. Dr. R. B. Bean: Preparations showing a racial peculiarity in the brain of the Negro.
3. Dr. J. L. Bremer: Models of a 4.0 mm. human embryo from the Harvard Embryological Collection.
4. Dr. Lydia M. DeWitt: Models of areas of Langerhans of certain mammals.
5. Professor Simon H. Gage: Preparations showing glycogen in animal tissues.
6. Susanna Phelps Gage: Plates illustrating the anatomy of a three-weeks human embryo, with especial reference to the forebrain.

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7. Dr. Ross G. Harrison: Sections showing the development of the peripheral nerves without sheath cells.
8. Dr. G. Carl Huber: Model of uriniferous tubule.
9. Dr. George S. Huntington: Types of bronchial and arterial distribution in the mammalian lung. (Morphological Museum of Columbia University.)
10. Dr. Wm. Keiller: *a.* Models showing the anatomy of the lymph nodes of the axilla. *b.* Casts showing the ligaments of the mammary gland.
11. Dr. Abraham T. Kerr: Specimens from the dissecting-room.
12. Dr. Franklin P. Mall: The chorion and decidua of a human ovum of the second week.
13. Dr. Charles S. Minot: A human embryo before the formation of the medullary plate, and other human embryos.
14. Dr. George L. Streeter: *a.* Preparations illustrating the histogenesis of the spinal ganglia. *b.* Apparatus for dissecting small objects.
15. Dr. John Warren: Models illustrating the development of the paraphysis, and pineal region in *Necturus*.
16. Dr. Burt G. Wilder: Exhibition of a manatee heart and fetus, and of the brains of a manatee, macroscelides, ceratodus, chimæra, and of a frog that lived five years after the removal of its cerebrum.

CONSTITUTION AND LIST OF OFFICERS AND MEMBERS.

CONSTITUTION.

ARTICLE I.

Section 1. The name of the Society shall be the "Association of American Anatomists."

Section 2. The purpose of the Association shall be the advancement of anatomical science.

ARTICLE II.

The officers of the Association shall consist of a President, two Vice-Presidents, and a Secretary, who shall also act as Treasurer. The officers shall be elected by ballot every two years.

ARTICLE III.

The management of the affairs of the Association shall be delegated to an Executive Committee, consisting of seven members, including the President and Secretary, ex-officio. One member of the Executive Committee shall be elected annually.

ARTICLE IV.

The Association shall meet annually, the time and place to be determined by the Executive Committee.

ARTICLE V.

Section 1. Candidates for membership must be persons engaged in the investigation of anatomical or cognate sciences and shall be proposed in writing to the Executive Committee by two members, who shall accompany the recommendation by a list of the candidate's publications, together with the references. The election shall take place in open meeting, a two-thirds vote being necessary.

Section 2. Honorary members may be elected from those not Americans who have distinguished themselves in anatomical research.

ARTICLE VI.

The annual dues shall be five dollars. A member in arrears for dues for two years shall be dropped by the Secretary at the next meeting of the Association, but may be reinstated, at the discretion of the Executive Committee, on payment of arrears.

ARTICLE VII.

Section 1. Five members shall constitute a quorum for the transaction of business.

Section 2. Any change in the constitution of the Association must be presented in writing at one meeting in order to receive consideration and be acted upon at the next meeting; due notice of the proposed change to be sent to each member at least one month in advance of the meeting at which such action is to be taken.

Section 3. The ruling of the Chairman shall be in accordance with "Roberts' Rules of Order."

ORDERS ADOPTED BY THE ASSOCIATION.

The election of delegates to the Executive Committee of the Congress of American Physicians and Surgeons shall take place every three years.

Newly elected members must qualify by payment of dues for one year within thirty days after election.

The maximum limit of time for the reading of papers shall be twenty minutes.

The Secretary and Treasurer shall be allowed his traveling expenses and the sum of \$10 toward the payment of his hotel bill, at each session of the Association.

That the Association discontinue the separate publication of its proceedings, and that the AMERICAN JOURNAL OF ANATOMY be sent to each member of the Association, on payment of his annual dues, this journal

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to publish the proceedings of the Association, including an abstract of the papers read.

Contributors of papers are requested to furnish the Secretary with abstracts within a fortnight after the meeting.

OFFICERS FOR 1905.

<i>President</i>	CHARLES S. MINOT.
<i>First Vice-President</i>	GEORGE A. PIERSOL.
<i>Second Vice-President</i>	J. MARSHALL FLINT.
<i>Secretary and Treasurer</i>	G. CARL HUBER.

Executive Committee.

LEWELLYS F. BARKER.....	Term expiring in 1905.
FREDERIC H. GERRISH.....	Term expiring in 1906.
GEORGE S. HUNTINGTON.....	Term expiring in 1907.
FRANKLIN P. MALL.....	Term expiring in 1908.
CHARLES R. BARDEEN.....	Term expiring in 1909.

*Delegate to Executive Committee of Congress of Physicians and Surgeons,
1903-1906.*

JOSEPH A. BLAKE.

Alternate.

FRANK BAKER.

*Delegate to the Council of the American Association for the Advancement of
Science.*

SIMON H. GAGE.

Member of Smithsonian Committee on the Table at Naples.

GEORGE S. HUNTINGTON.

For addresses of officers, see list of members.

Honorary Members.

JOHN CLELAND.....	Glasgow, Scotland.
JOHN DANIEL CUNNINGHAM.....	Edinburgh, Scotland.
MATHIAS DUVAL.....	Paris, France.
CAMILLO GOLGI.....	Pavia, Italy.
ALBERT VON KÖLLIKER.....	Würzburg, Germany.
ALEXANDER MACALISTER.....	Cambridge, England.
L. RANVIER.....	Paris, France.
GUSTAV RETZIUS.....	Stockholm, Sweden.
CARL TOLDT.....	Vienna, Austria.
SIR WILLIAM TURNER.....	Edinburgh, Scotland.
WILHELM WALDEYER.....	Berlin, Germany.

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XXIV Proceedings of the Association of American Anatomists

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Proceedings of the Association of American Anatomists XXV

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XXVI Proceedings of the Association of American Anatomists

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SUPPLEMENT

TO

VOLUME IV

THE AMERICAN JOURNAL OF ANATOMY
BALTIMORE, MD., U. S. A.

JUNE 1, 1905

ON THE DEVELOPMENT AND SHAPE OF URINIFEROUS
TUBULES OF CERTAIN OF THE HIGHER MAMMALS.

BY

G. CARL HUBER.

From the Histological Laboratory of the University of Michigan.

WITH 24 FIGURES.

As is well known, the excretory system of the amniota develops as a series of distinct organs, the pronephros, the mesonephros, and the metanephros. The pronephros, which is the first excretory organ to differentiate, and is also phylogenetically the oldest, disappears in all amniota; its consideration is here dispensed with. The mesonephros, which functionates throughout life as the chief excretory organ of anamnia, is an embryonic organ in amniota, in which it disappears as an excretory organ and is replaced by the permanent or true kidney, the metanephros. The development of the metanephros is, however, so closely related to that of the mesonephros, in both its phylogeny and ontogeny, that a consideration of the development of the former will to some extent necessitate a consideration of the development of the latter. This will be done only so far as necessary, as a consideration of the development of the mesonephros will not form a part of this contribution.

Our present day conception of the anlage and development of the metanephros dates from Kupffer's contribution based on observations made on sheep embryos. In an embryo 8 mm. in length he found that from the dorsal wall of the Wolffian or mesonephric ducts near their posterior termination, there is formed an evagination which he designated as the "Nierenkanal." These buds, one of which appears in connection with each Wolffian duct, grow dorsally and cephalad and, as observations on older embryos revealed, become associated with the development of the permanent kidney. Anticipating these observations, we find those of Remak and Kölliker, who recognized these buds, but traced their origin to the cloaca or bladder, and of still earlier date the observations of Rathke, who recognized a blastema situated between the dorsal wall of the embryo and the mesonephros in which the kidney had its origin and from which the ureters were supposed to grow toward the bladder. That the

permanent kidneys have their origin from buds which arise from the Wolffian ducts is now very generally accepted, Kupffer's observations having been widely confirmed and extended to embrace the different classes of the amniota. While, as just stated, there is a unanimity of views concerning the anlage of the permanent kidney, the views concerning the mode of development and the histogenesis of the tubular and other structural elements are still at variance.

According to Remak and somewhat later Kölliker, the development of the permanent kidneys, after their anlage in the buds arising from the Wolffian ducts, proceeds in a manner similar to that observed for other tubular and for alveolar glands, or, as stated by these observers, after the manner of the development of the lungs. The epithelial kidney anlage was said to grow forward and to present an anterior vesicular enlargement, which soon elongates, the anlage differentiating into ureter and primitive kidney pelvis. On this, solid buds and ampullæ make their appearance, which in their further growth enlarge and elongate and obtain a lumen, thus forming anlagen for the papillary ducts. These in their further growth undergo division and give origin to hollow buds which form the anlagen for the convoluted (secretory) portions of the uriniferous tubules and the epithelial portions of the Malpighian corpuscles. According to this view the collecting ducts and coiled uriniferous tubules are developed by direct budding from the epithelial renal anlage derived from the Wolffian duct.

On the other hand, Kupffer described a discontinuous origin for the uriniferous tubules. In a sheep embryo of 10 mm. length, he observed a group of cells, clearly differentiated from the surrounding tissue and in close relation with the renal anlage—Nierenkanal—in which he recognized the anlage of the kidney (figured in Fig. 4, Pl. XV of his article). In this group of cells Kupffer recognized three zones—a zone consisting of compactly arranged cells in close relation to the renal anlage; a middle zone of less compactly arranged cells, and an outer zone in which connective tissue fibers were observed. In a sheep embryo of 15 mm. length, a differentiation in the middle zone was observed in that the cells were arranged in twisted cords ("Zellen sich in gewundene Streifen ordnen") which were as yet not clearly differentiated. The cells of these twisted cords were not connected with the renal anlagen. These cords were interpreted as the anlagen of the coiled uriniferous tubules. The darker zone of cells immediately surrounding the renal anlage was thought to contribute to their further development. Kupffer could not exclude the possibility that perhaps later generations of tubules had an origin which differed from that here given. According to

this view, the coiled uriniferous tubules have their origin in a tissue which is distinct from that of the renal anlage. Kupffer's observations though faulty in many respects, as later investigations have shown, must be recognized as of fundamental importance, as concerns both the phylogenetic and the ontogenetic development of the permanent kidney.

Nearly all investigators who, since the appearance of Kupffer's observations, have considered the question under discussion, have adopted one or the other of the two views above hastily sketched and, even taking into consideration the most recent contributions to this subject, the statement seems warranted that the problem under discussion is still awaiting final solution. Schreiner, in a recent most admirable contribution to this subject, especially as concerns the earliest stages of the development of the permanent kidney, has grouped under respective heads all the more important contributions dealing directly or indirectly with the development of the permanent kidney, and, as it is not my purpose to enter extensively into a discussion of all the literature bearing on the topic under consideration since this has been undertaken by Rückert, Herring and Schreiner, I have adopted and extended the above mentioned classification of Schreiner. So far as accessible to me (the exceptions I have noted), I have critically reviewed the literature to which reference is here made. It should, however, be stated that, while adopting such a classification, I must regard it as somewhat forced. A number of the contributions here referred to and especially certain of the earlier ones, require interpretation through a view-point gained by familiarity with more recent investigations and with actual preparations before a classification of them can be made.

Among investigators who adhere to the view that the tubules of the permanent kidney are developed by a direct budding from the epithelial renal anlage derived from the Wolffian duct after the manner of other tubular and of alveolar glands may be mentioned:—Remak, 55; Kölliker, 61; Colberg, 63; Waldeyer, 70; Toldt, 74; Pye, 75; Löwe, 79; Ribbert, 80; Hortolès, 81; Kallay, 85; Janosik, 85; Nagel, 89; Golgi, 89; Minot, 92; Haycraft, 95; Schultz, 97; Kollmann, 98; V. Ebner, 99; Gerhardt, 91; Stoerk, 91; Strahl, 92; Disse, 92; Stoerk, 94. Stoerk's fuller publication gives no additional data as to his view of the origin of the uriniferous tubules and I assume that he still adheres to the views expressed in his earlier contribution.

The investigators who have followed Kupffer in assuming a separate and distinct origin for the coiled uriniferous tubules may be arranged as follows:

Bornhaupt, 67; Thayssen, 73; Riedel, 74; Balfour, 76; Braun, 78;

Fürbringer, 78; Emery, 83; Wiedersheim, 90; Hamburger, 90; Weber, 97; Chievitz, 97; Ribbert, 99; Herring, 100; Schreiner, 102; Haugh, 103; Keibel, 103; Felix, 104. These observers, while expressing a unanimity of view concerning the separate anlage of the tubular system of the permanent kidney, are not in accord as to the histogenesis of the tissue from which the tubules are differentiated.

Certain observers, among whom may be mentioned Sedgewick, 80; Riede, 87; Hoffmann, 89; Gregory, 100, may be separately grouped, since they assume an intermediate position inclining toward a separate anlage of the tubules but assuming a histogenetic relationship between the tissue from which the tubules are developed and the epithelial renal anlage. This brief array of the contributors to the literature under consideration may serve as an introduction to a fuller discussion of certain of the more recent contributions; these and others will receive further mention in presenting the results of my own investigations.

Of the recent contributions to our knowledge of the development of the permanent kidney, the article of Schreiner deserves special mention. The results presented are based on observations made on representatives from the different classes of amniota and embrace a study of the origin and development of the tubules of the Wolffian body and of the anlage of the different constituent elements of the permanent kidney. I find it somewhat difficult to give a brief summary of this article, which is accompanied by numerous illustrations of sections, of profile reconstructions and of reconstructions after the Born method. The discussion of his own observations, he begins for each type investigated with a consideration of the histogenesis of the tubules of the Wolffian body, following this by a discussion of the origin and development of the permanent kidney in each type. Schreiner traces the origin of the tubules of the Wolffian body to a cell-mass which he designates as the *nephrogenic tissue* (a term suggested by Rabl), which is identical with the blastema of other authors. This nephrogenic tissue has its origin in the intermediate cell-mass and extends as an unsegmented cord along the mesial and dorso-mesial side of the Wolffian ducts to their termination in the cloaca. From the cells of this cord of cells, which increase in number by division, are differentiated the tubules of the Wolffian body. These appear first in the anterior segments and, as development proceeds, also in the posterior segments. The differentiation of the tubules of the Wolffian body does not, however, extend to the posterior limits of the nephrogenic tissue, but ceases a number of segments anterior to the termination of the Wolffian ducts and nephrogenic tissue. The permanent kidneys have their origin in evaginations (one for each side) from the dorso-mesial wall of the Wolffian ducts not far from their termination in the cloaca. These evaginations grow dorsally into the posterior portion of the nephrogenic tissue and in doing so become capped with the nephrogenic tissue. The evaginations, or as they are known, the renal anlagen, renal ducts or Nierengänge, at first present a bulbous extremity. This elongates in an antero-posterior direction and develops buds and ampullæ. In the meantime, the nephrogenic tissue, which at first surrounds the bulbous extremity of the renal anlagen as a compact cell-mass, breaks up into several cell-masses, each of which caps one of the primary branches of the renal anlagen, at the same time losing its connection with the nephrogenic tissue from which are developed the tubules of the Wolffian body. This portion of the nephrogenic tissue Schreiner now terms the metanephrogenic tissue in contradistinction to the mesonephrogenic tissue. The metanephrogenic tissue, in the majority of the forms studied by Schreiner may be differentiated more or less clearly into an inner zone composed of cells more compactly arranged and presenting other characteristic features and an outer zone, the cells of which are less compactly arranged and approach in appearance mesenchymal tissue. Schreiner further shows that the epithelium of the ureters and pelvis of the kidney as also the collecting tubules to their terminations are developed from the renal anlagen (Nierengänge), while the secretory portion of the uriniferous tubules from their termination in the collecting tubes to and including the epithelial portion of Bowman's capsule are differentiated from the inner zone of the metanephrogenic tissue, the interstitial tissue and the capsule of the kidney developing from the outer zone. Schreiner's observations, accompanied by a very full discussion of the literature and substantiated by numerous figures, appear to argue conclusively for a separate anlage of the tubules of the Wolffian body and the secretory portion of the

uriniferous tubules. I regard it as a distinct achievement on his part to be able to trace the close relationship between the mesonephrogenic tissue from which are developed the mesonephric tubules, the separate anlage of which is generally accepted, and the metanephrogenic tissue from which are developed the secretory portions of the uriniferous tubules of the permanent kidney. As is no doubt evident, the nephrogenic tissue here referred to has long been known as the renal blastema: there are, however, various opinions regarding its origin and its share in the development of the kidney. I shall have occasion to make further reference to this excellent article of Schreiner.

Herring, in a very creditable contribution based on observations made on material derived from human embryos, was first led to believe that the kidney tubules were branches of the collecting tubes, but "could never find the early stages, which should have been easily seen if that view were correct." He further states that "in the thick layer of the capsule, cells are seen which show a gradual transition in appearance from embryonic connective tissue cells to a character resembling that of the epithelial cells of the early convoluted tubules. Between these cells and those of the ampullæ" (dilated ends of the collecting tubes) "there is always a distinct line of separation and in the majority of instances there is a space where the tubule has shrunk during the process of hardening. The careful examination of serial sections has convinced me that these masses of cells which appear under the capsule and in the interlobular septa, are quite independent of the ureter branches and give rise to the Malpighian bodies, convoluted tubules and Henle's loop, uniting always with short branches from the ampulla."

Hamburger's paper, based largely on investigations made on the kidneys of mouse embryos and young mice, contains important data concerning the later stages of the development of the uriniferous tubules and the development of the Malpighian pyramid. His observations are based on serial sections and on what must be regarded as very successful preparations made by maceration and teasing. He makes only incidental mention of the origin of the uriniferous tubules, which he regards as developing from small bodies (*Körperchen*), the smallest of which are spherical cell groups which have only an apparent connection with the enlarged distal ends of the collecting ducts.

Haugh, in one of the most recent contributions to the subject, in which he presents observations made on an extensive material derived from human embryos of all ages, gives a full account of the development of the pelvis of the kidney and of the shape of the pelvis of embryonic and adult kidneys, based on metal injections and on wax reconstructions. He also presents data concerning the lobulation of the human kidney and on the development of the collecting tubules. He treats, however, very superficially—to use his own words—the earlier stages of the development of the uriniferous tubules, allying himself with those authors who defend a separate anlage of the uriniferous tubules.

Stoerk, on the other hand, defends the older view of the development of the uriniferous tubules. According to this observer, the renal anlage, derived from the Wolffian duct, grows dorsally and, after repeated dichotomous division, meets the anlage of the renal capsule which retards the further radial growth of the tree-like branches of the renal anlage. Each of the branches now develops an ampullar enlargement at its extremity, which later assumes a heart-shape. Each half of the heart-shaped enlargement buds out laterally and forms a short tube, the whole structure now assuming the appearance of a Y. Each arm of the Y-shaped termination then divides dichotomously, the one branch, which for a time remains small, passing toward the periphery, the other, which grows more rapidly, budding and growing toward the stem of the Y. The Y-shaped structure now presents the appearance of an inverted anchor. The points of this anchor-shaped structure now grow in such a manner as to assume the shape of an S. Such an S-shaped structure is an anlage of a uriniferous tubule and of Bowman's capsule. Stoerk's more recent contribution contains only a brief statement concerning the anlagen and early developmental stages of the uriniferous tubules and this is merely a reiteration of the views presented in his earlier paper. In this second paper, Stoerk gives the results of observations made on serial sections of material derived from human embryos and numerous wax-reconstructions made therefrom. These reconstructions represent mainly the earlier developmental stages of the uriniferous tubules, a fact which is no doubt responsible for certain errors which he has committed, in using the data gained by a study of the earlier stages to interpret the shape and structure of more fully developed tubules. As I shall have occasion to refer frequently to this paper in connection with a discussion of my own results, its further consideration may here be dispensed with.

Gerhardt, who has recently published, from O. Hertwig's laboratory, also defends the view that the uriniferous tubules are developed by budding and that the permanent kidney is an organ *sui generis*. As material, he used largely mouse embryos, but also those of chicken, dog, and pig. Gerhardt's article is concerned largely with a discussion of the literature and with theoretic speculations. His own observations are very briefly given and, as illustrations are wanting, may be summarized by giving one of his conclusions: "The peripheral portions of the uriniferous tubules arise through a continuous growth of the collecting tubes. It cannot be demonstrated that in the cortex formed tubes make secondary connection with the tubules of the medulla." The capsule of the glomeruli he regards as an invagination of the distal end of the uriniferous tubules.

In Chapter IV (Entwickelungsgeschichte und Missbildungen der Nieren) of Vol. 52b, Deutsche Chirurgie (Küster, Krankheiten der Niere), written by Strahl, this observer

states that so far as his own observations go, he is inclined to accept a continuous epithelial anlage of the uriniferous tubules as given by Minot and Nagel; he does not, however, desire to commit himself until he himself has made further investigations. A number of excellent illustrations are found in this chapter.

Disse, who discusses briefly the development of the kidney in his account of the anatomy of the urinary organs as given in Bardeleben's Handbuch, states that as a result of his own observations made on guinea pig and pig embryos, he is led to conclude that both the straight and the coiled uriniferous tubules have the same anlage, namely from the hollow buds of the primitive kidney pelvis. The selection of the section which forms the basis for his Fig. 77, showing the anlage of the coiled uriniferous tubule seems to me unfortunate. The S-shaped portion (anlage of the coiled tubule) is not cut through the center of its lumen, only a small part of which is seen in the figure. The apparently continuous structure as represented might, it seems to me, be simulated by an overlapping of the two bevelled surfaces of two discontinuous structures (anlage of coiled tubule and collecting duct), especially as the section from which the drawing was made seems to have been relatively thick.

Haycraft's contribution to the development of the kidney of the rabbit may yet receive consideration as his observations have been widely accepted. He states that "the tubules and Malpighian bodies arise as buds of solid cells from the wall of the primary renal vesicles" (ampullar enlargement of the primary divisions of the renal anlage) "close to the cortex. The bud makes a double bend like an S, first turning with a large sweep away from the vesicle, then turning toward it and sharply away again. The basement membrane can be traced along it and a central lumen forms almost to its tip." Haycraft also traces the development of these S-shaped buds into the uriniferous tubules and gives his conception of the form of such a tubule as present in the adult kidney of rabbits. The figures accompanying this article are of special interest to me, since my series of sections of rabbit embryos of various ages as also numerous reconstructions of uriniferous tubules made from the same, afford a basis for their interpretation. His Fig. 8, "A high power view of the first formation of a urinary tubule from a primary renal vesicle" represents, I fear, a much older stage, only a portion of this tubule being shown in the section sketched. His Fig. 10, I interpret as embracing parts of at least two tubular anlagen, there sketched as one tube, owing to the position given to the glomerular anlage with reference to the other portions of the tube. Further reference to this article will be made later.

Brief mention may yet be made of the conclusions reached by a number of observers who have investigated the development of cystic kidneys. Hildebrand suggested that the want of union between the collecting tubules and the anlagen of the uriniferous tubules was the cause of the cysts of the congenital cystic kidney, in that, when this condition obtains, as "the glomeruli begin to functionate there is no opportunity for the outflow of the secretion and consequently the tubules are expanded into cystic structures." Ribbert, who describes observations made on a cystic kidney of a new-born male child, was able to show that many of the cysts were developed from Malpighian corpuscles which showed no connection with collecting tubules, the ends of certain of which were also cystically enlarged. Meyer found in microscopic sections of the kidneys obtained from a 9-weeks-old female child, which showed other congenital defects, certain regions where the kidney parenchyma was normally developed and other regions where collecting tubules and Malpighian corpuscles were observed, but where the tubular portions of the uriniferous tubules which normally unite these structures are wanting and in place of which there was observed "an undifferentiated tissue rich in cellular elements in which the cells only in certain regions were arranged in the form of rings or cords." These observations, it appears to me, confirm in a satisfactory way the conclusions of observers, who recognize separate anlagen for the collecting tubules and the uriniferous tubules proper.

From this brief summary of the literature, it may be seen that the controversy found in the early literature concerning the origin and development of the coiled portion of uriniferous tubules of the permanent kidney is still found in the more recent literature, the weight of evidence, however, being on the side of a discontinuous origin of the coiled portion of the tubule, as Félix has very recently correctly stated.

This investigation was begun in the spring of 1902. In considering the possible sources of error of other investigators who have dealt with this subject, it occurred to me that the thickness of the sections studied might alone be responsible for a certain per cent of the discrepancies found in the literature. The average thickness of serial sections of embryos used in embryological investigations is seldom below 10 μ , more often perhaps 20 μ . My own observations soon convinced me that

sections having a thickness of 10 to 20 μ were not suitable for interpreting the early stages of kidney development, as in some sections fields were found which might be used respectively for demonstrating either a continuous or discontinuous origin of the coiled uriniferous tubules. It was further hoped that a solution of the question might be obtained by making free use of reconstruction methods, especially of the Born wax-plate method. The wax-plate method of reconstruction had previously been used in the study of the development of the kidney by Chievitz. He gives three figures, which are, however, somewhat difficult to interpret, owing to the fact that the models were not completed by smoothing over the irregularities which always result when the cut-out portions are placed together. Since my own investigation was begun and in part completed, there have appeared three contributions based in part or as a whole on data gained by reconstructions. Mention has been made of the work of Schreiner, who, however, studied, among other questions, only the anlage of the coiled tubules and of this portion of his work he gives no reconstructions. Haugh's reconstructions are confined almost wholly to such of the pelvis and straight collecting tubules of kidneys of relatively young human embryos. It is rather to be regretted that Haugh did not devote more time to the finishing of his wax models, which would have enabled him to give clearer illustrations of the models made, than accompanying his otherwise excellent article. The most recent contribution to this subject, that of Stoerk, is based largely on results obtained by means of wax-plate reconstructions, the value of which method he clearly recognizes. The contributions of Schreiner, Haugh, and Stoerk which, as stated, have appeared since my own work was in progress, have been of material assistance to me in formulating my own views. They do not, however, as appears to me, make superfluous the publication of my own results. These are confirmatory, as will appear, of that portion of Schreiner's work which deals with the anlage of the metanephros and its tubules; Haugh touches only incidentally on the development of the coiled uriniferous tubules; and finally my own observations have led me to conclusions which differ materially in a number of particulars from those reached by Stoerk.

The investigation, as projected, included not only a study of the anlage of the uriniferous tubules and their mode of development, but also a study of the form of the more fully developed uriniferous tubule, if possible by means of wax-plate reconstruction. That this seemed desirable, requires, I believe, no argument. A portion of the results here given were presented at the December meeting of the Association of American

Anatomists in 1903, accompanied by a demonstration of a portion of the models here figured.

MATERIAL.

In selecting the material to be used, it seemed to me desirable to include in this study developmental stages of both simple and lobulate kidneys; the embryos selected depended largely on the accessibility of the material. The embryological material used in the investigation was as follows:

HUMAN EMBRYOS.

NUMBER.	LENGTH OR AGE OF EMBRYO.	FIXATION.	REMARKS.
1	About 15 days old	Formalin	Received from Dr. Chivers of Jack., Mich. (Not well preserved).
2	10-mm. neck-breech	Alcohol	Received from Dr. Steiner of Lima, Ohio.
3	15-mm. neck-breech	Müller's fluid	Received from Dr. Roberts, Buffalo, N. Y.
4	18-mm. head-breech	Formalin	Received from Dr. Peterson, Ann Arbor.
5	20-mm. head-breech	Formalin	Received from Dr. Peterson, Ann Arbor. Model, Fig. 1 (B)
6	22-mm. head-breech	Formalin
7	3-cm. head-breech	Formalin	Received from Dr. Hickey, Model, Fig. 9.
8	4-cm. head-breech	Formalin	Received from Dr. Hickey.
9	5.8-cm. head-breech	Formalin
10	6-cm. head-breech	Müller's fluid
11	6.5-cm. head-breech	Formalin	Received from Dr. Hickey, Model, Fig. 10.
12	13-cm. head-breech	Formalin	Received from Dr. F. C. Wright, then of Calumet, Mich.
13	14.5-cm. head-breech	Formalin	Received from Dr. Darling, Ann Arbor.
14	14.5-cm. head-breech	Formalin	Received of Dr. F. C. Wright, then of Calumet, Mich.
15	18.5-cm. head-breech	Formalin	Received of Dr. F. C. Wright, then of Calumet, Mich.
16	Early part of 7th mo.	Bichloride	Dr. Warthin, Ann Arbor, only the kidneys obtained. Models, Fig. 4.
17	27 cm. in Length	Zenker's and Orth's fluids	Dr. C. S. Minot, Boston. Only the kidneys available.
18	Child less than week old	Formalin
19	Child 10 days old	Bichloride

CAT.

NUMBER.	LENGTH OR AGE OF EMBRYO.	FIXATION.	REMARKS.
1	9-mm. neck-breech	Bichloride
2	13-mm. head-breech	Bichloride	Model, Fig. 1 (A).
3	20-mm. head-breech	Müller's fluid
4	22-mm. head-breech	Bichloride	Models, Fig. 6.
5	3-cm. head-breech	Formalin
6	4-cm. head-breech	Formalin	Model, Fig. 16 (A).
7	4.5-cm. head-breech	Formalin
8	6-cm. head-breech	Formalin	Model, Fig. 16 (B).
9	9-10-cm. head-breech estimated as just before birth	Zenker's fluid	Models, Fig. 18 (A) and Fig. 20.
10	Kitten 1 month old	Zenker's and Tellyesnický's fluids
12	Kitten $2\frac{1}{2}$ months old	Zenker's and Tellyesnický's fluids
14	Full grown cat	Zenker's and Tellyesnický's fluids

RABBIT.

1	9-mm. neck-breech	Bichloride
2	14-mm. head-breech	Zenker's Fluid	Models, Fig. 5, A, E, F.
3	18-mm. head-breech	Bichloride	Models, Fig. 5, B, C, D.
4	20-mm. head-breech	Tellyesnický's fluid
6	22-mm. head-breech	Alcohol-acetic
7	29-mm. head-breech	Alcohol-chloroform-acetic
8	3.5-cm. head-breech	Zenker's fluid	Models, Fig. 14.
9	4-cm. head-breech	Zenker's fluid
10	4.3-cm. head-breech	Tellyesnický's fluid
11	4.5-cm. head-breech	Müller's fluid
12	4.8-cm. head-breech	Formalin
13	6.5-cm. head-breech	Formalin	Models, Fig. 18 (B) and Fig. 19 (A).
14	Rabbit one day old	Zenker's fluid	Model, Fig. 19 (B).

PIG.

1	5-mm. neck-breech	Sublimate-acetic
2	10-mm. neck-breech	Sublimate-acetic
3	14-mm. neck-breech	Chromic acid
4	18-mm. head-breech	Graaf's fluid
5	20-mm. head-breech	Picro-sulphuric
6	23-mm. head-breech	Chromic acid
7	24-mm. head-breech	Alcohol-acetic	Model, Fig. 17, B.
8	28-mm. head-breech	Formalin	Models, Fig. 17, A and C.
9	3-cm. head-breech	Formalin
10	3.5-cm. head-breech	Formalin
11	4.4-cm. head-breech	Formalin
12	5-cm. head-breech	Formalin

METHOD.

Of the various fixing fluids used, fixation by Zenker's fluid proved most satisfactory.

The wax-plate reconstruction method was largely used in gaining the data which form the basis for this contribution. It was found that the material could be most readily manipulated in this way by using serial sections $5\ \mu$ in thickness and a magnification of 400 diameters, necessitating the use of wax-plates 2 mm. in thickness. As already stated, it is often exceedingly difficult in sections more than $5\ \mu$ in thickness to interpret with any degree of clearness the earlier stages in the development of the kidney tubules; the same may be said of sections from more fully developed kidneys, where the tubules are already well differentiated. The difficulty here met with is that even in sections having a thickness of not more than $10\ \mu$, the tracing of a tubule through a series of sections is often made difficult by the fact that portions of two tubules lying over each other—with reference to the plane of the section—and in contact are both included in the same section and appear as one tubule, thus easily leading the observer astray. For the earlier stages—embryos having a length of about 1.5 cm.—the entire embryo was cut in sagittal serial sections; for embryos measuring from 1.5 to 2.5 cm., only the posterior half was thus cut, and for older stages, the kidneys were removed after fixation and cut in series either longitudinally or transversely. The difficulty of obtaining unbroken series of sections $5\ \mu$ in thickness, without wrinkling or distortion when mounted, was met for many of the series by cutting the sections one by one on an ordinary sliding microtome—Altmann pattern—with the knife at an angle of about 45° and with the knife blade covered with a layer of distilled water while cutting. This latter procedure I have found very useful in cutting thin paraffin sections. The sections are transferred as cut to an Esmarch dish filled with distilled water in which they float and flatten out. They are arranged as cut on a slide covered with albumin fixative placed at an angle in the same dish. When the slide has been filled with sections, it is placed on the warm oven until the water evaporates. The sections are then ready for staining. This procedure, though much more time-consuming than when the sections are cut with an automatic microtome, seemed to present advantages which compensated for the additional time necessary for its prosecution; especially was this true for the older stages, in which the kidneys alone were cut. The sections were stained on the slide in Ehrlich's haematoxylin and counterstained mainly in eosin or erythrosin, the latter giving the better differentiation and sharper contrast than the other protoplasmic stains used.

The outline drawings used in making the models were sketched with the aid of the camera lucida, a comparatively easy procedure for the earlier stages, very time-consuming for the later stages, where constant shifting of the field was necessary. The steps used in making the models were those generally used when the Born wax-plate method of reconstruction is employed. Special orienting planes or lines were generally dispensed with, as it was found practicable to use for this purpose certain prominent structures within the sections. The reconstruction of the earlier stages of kidney development—the primary renal anlage and renal pelvis with its primary branches, also the earliest developmental stages of the uriniferous tubules—is a comparatively simple procedure. The same can, however, not be said of the older stages and more fully developed tubules. The complicated and varied arrangement of the proximal and distal convoluted portions of the more fully developed uriniferous tubules, above all the length of the loop of Henle make the tracing of a single tubule through a series of sections a matter of difficulty. However, on careful search through a series of sections, instances would be found where one or the other arm of Henle's loop was cut through nearly its entire length. By using this as a starting point, it was generally possible to trace the remainder of the respective tubule. In doing so, it was found helpful to make profile or temporary wax reconstructions of certain parts of the tubule or to make temporary reconstructions of all the tubules of a given area supposed to contain the portions of the tubule sought and in this way separate it from surrounding tubules. After thus mapping out a given tubule in its entirety, drawings were made which could be used for reconstruction of the whole tubule.

ANLAGE AND EARLY STAGES OF DEVELOPMENT OF THE METANEPHROS.

The earlier stages of the development of the permanent kidney, the anlage and early development stages of the renal evagination and of the nephrogenic tissue (kidney blastema) shall here be considered very briefly, since the appearance of Schreiner's contribution obviates the necessity of a fuller discussion of this portion of my investigation. Neither does it seem necessary to reproduce by way of illustrations the numerous models made in the earlier part of this work showing shape, size, and relationship of the renal evagination as found in the cat, rabbit, pig, and man, nor to discuss the appearances presented by them during the earlier developmental stages, since such figures would in nearly every instance duplicate the figures of similar stages given by Schreiner (rabbit, text-figure 15-21; human, text-figure 26-27; pig, text-figure 29-30 of his article) based on profile and wax reconstructions, except for the

cat; this animal he did not study, but it presents no special features. Schreiner considers very fully the anlage and early developmental stages of the kidney of the rabbit. A brief review of his account may here be given. In the rabbit, the renal evagination arises in the 31st segment (about the 11th day, Kölliker, Minot) from the dorso-median wall of the Wolffian duct near its termination in the cloaca and grows into a tissue known as the nephrogenic tissue, which soon surrounds its blind end as a cap of hemispheric shape. As concerns the renal evagination, Schreiner's and my own observations confirm those of other investigators, who, since the appearance of Kupffer's contribution, have studied the anlage of the kidney in amniota. Opinions differ, however, as concerns the histogenesis of the tissue generally known as the renal blastema here designated as the nephrogenic tissue and as to the rôle it plays in the development of the kidney. The general statement may be made that observers who regard the kidney tubules as developing by a process of budding, interpret the nephrogenic tissue as of mesenchymal origin, destined to form the capsule, interstitial connective tissue and vascular sheaths. Concerning it Minot states that "the histogenesis of the mesenchymal portion of the kidney is almost unknown." The majority of those observers who accept a separate origin for the kidney tubules have traced their origin to a special tissue (nephrogenic tissue, renal blastema) the histogenesis of which may here be considered, although in doing so it will be necessary to anticipate certain facts which will be considered more fully later. This seems justified, since the tissue in question makes its appearance before the renal evaginations are present.

Braun, in his account of the development of the urogenital system of reptiles (lizards), described irregular cords of cells (*Nierenzellenstrang*, generally known as Braun's cords), whose origin he traced to the colomic epithelium and which were thought to be the anlage of the uriniferous tubules. Minot regards these cords "as merely the beginning of the condensed mesenchyme of the renal anlagen," and Schreiner regards them as identical with the cell-mass designated by him as nephrogenic tissue. Wiedersheim, in his account of the development of the urogenital apparatus of crocodile and turtle embryos, confirms in part Braun's observations, differing from him in that he regards the caudal end of the mesonephros as the seat of origin of the cell-masses or cords from which the kidney tubules are developed. He recognizes further a condensed mesenchyme which surrounds the metanephric duct and which he characterizes as staining more deeply than the surrounding mesenchyme with which it blends. This proliferating cell-mass, which, as he states, is also found in other amniot embryos can not alone be regarded as the "metanephros blastema," as it in itself does not give origin to the strictly glandular parts of the kidney, but the interstitial connective tissue and the vessel sheaths. Sedgwick, who gave a very clear account of the anlage of the mesonephros and metanephros as observed in chick embryos, traces the origin of the Wolffian tubules to a cell-mass designated by him the Wolffian blastema, which has its origin in the intermediate cell-mass. As described by him, the Wolffian blastema extends as far back as the 34th segment, that is, to the opening of the Wolffian duct into the cloaca. This Wolffian blastema breaks up into the Wolffian tubules as far back as the 30th segment. He states further that "behind this point it remains for some time almost or quite passive and ultimately gives rise to the epithelium of the permanent kidneys. In consequence of this, I have called that part of the Wolffian blastema between the 31st and 34th segments the kidney blastema," and again "that it is important to notice that this kidney blastema develops in exactly similar manner to the Wolffian blastema. It is not until the fourth day, when the ureters have appeared that it is possible to draw a line between the two." "In yet older embryos, in which the ureter is more fully developed and overlaps the hind end of the Wolffian body, the kidney blastema has quite broken off from the Wolffian body and invests the anterior end of the ureter." I have quoted thus fully

from Sedgwick's paper, because he is the first to give a clear account of the histogenesis of the tissue into which grows the renal evagination. Ribbert, in a recent contribution, also discusses the origin of the tissue (which he characterizes as a differentiated cell-layer), which surrounds the blind end of the epithelial renal anlage, but gives no definite conclusions. Certain it is, he states, that the ureters grow into a dense cell-cord, which may be regarded as the distal end of the mesonephros, as is shown in his Fig. 4, combined from three sections of a series of sections of a Guinea-pig embryo. The histogenesis of the nephrogenic tissue (renal blastema) has been very fully considered by Schreiner, as has been stated in a brief review of his work given on page 4. For all types studied by him, he was able to show very conclusively that the nephrogenic tissue of the metanephros in common with that of the mesonephros had its origin in the intermediate cell-mass and extended as an unsegmented cord of cells through the regions in which is developed the mesonephros to where the Wolfian duct terminates in the cloaca. In the region of the mesonephros, this tissue gives origin to the mesonephric tubules; posterior to the mesonephros, it becomes associated with the development of the metanephros, forming the nephrogenic tissue, which surrounds the blind end of the epithelial renal evagination, as was shown for the chick by Sedgwick. The above quotations were selected with a view of presenting the various views held concerning the histogenesis of the nephrogenic tissue.

We may now resume a consideration of the anlage and early developmental stages of the metanephros as observed in the rabbit. The renal evagination, which, as stated, buds dorsally into the nephrogenic tissue, in its further growth extends in a dorsal direction (the account here given follows Schreiner) pushing with it the nephrogenic tissue. This now assumes a dorso-median position with reference to the Wolfian duct, instead, as before, of lying in contact with it. In its growth, the renal evagination develops an enlarged blind end, which may be known as the primary renal pelvis and a stalk which may be designated as the ureter. The nephrogenic tissue, which surrounds the primary renal pelvis differentiates into an inner zone composed of epithelioid cells, which immediately surround it and an outer zone which presents the appearance of a condensed mesenchyme, which shows no distinct demarcation toward the surrounding mesenchyme; the two zones form the metanephrogenic tissue which has become separated from the mesonephrogenic tissue. As development proceeds, the primary renal pelvis grows in a dorsal and cephalad direction and elongates in an antero-posterior direction; at the same time, it begins to rotate on its axis in such a way as to make its dorsal surface have a more lateral position. The ureter likewise elongates and curves cephalad. The metanephrogenic tissue with its inner and outer zones is clearly recognized and surrounds the primary renal pelvis as a continuous layer. In slightly older embryos, the primary renal pelvis reaches a position dorsal to the posterior end of the mesonephros. In shape it has become still more elongated and presents two slightly enlarged ends with a narrower middle portion from which arises the ureter; it shows thickenings and buddings in its wall which in slightly older stages are clearly recognized as evaginations, the anlagen of the primary branches of the primary renal pelvis. The primary renal pelvis now consists of a narrower middle portion, from which arises the ureter and two enlarged ends and from it and more particularly from

the narrowed middle portion, there spring three pairs of branches, each of which, as soon as clearly differentiated, presents a short stalk and a bulbous end. The entire metanephros now has a position which is dorsal to the hind end of the mesonephros. The metanephrogenic tissue, which, during the earlier stages, formed a continuous layer entirely surrounding the primary renal pelvis, presents a different disposition with the appearance of the primary branches and this applies more particularly to its inner zone, which, while it shows the structural appearances observed in earlier stages, presents a characteristic distribution. On the appearance of the primary branches, the inner zone of the metanephrogenic tissue no longer surrounds the entire primary renal pelvis, but only its enlarged extremities and the bulbous ends of its branches, the narrowed middle portion and the stalks of the branches being free from it. The inner zone of the metanephrogenic tissue is clearly recognized both by its staining reactions and by its structural characteristics. The primary renal pelvis and its branches, as also the inner zone of the metanephrogenic tissue, are surrounded by a tissue that stains less deeply than the inner zone and presents fewer nuclei, the outer zone of the metanephrogenic tissue. The entire renal anlage is surrounded by mesenchymal tissue, containing relatively few nuclei, these having a more or less concentric arrangement. For a fuller discussion of the anlage and early developmental stages of the renal evagination and the accompanying changes presented by the nephrogenic tissue as observed in the rabbit, the reader is referred to Schreiner's paper (pages 98-121). My own observations confirm his results in full except as concerns the anlage of the metanephrogenic tissue, where the necessary stages are wanting in the material at my disposal. A separate discussion of the anlage and early developmental stages as observed in cat, pig, and human embryos does not seem necessary here, as this would involve unnecessary repetition, since the types studied present great similarity in the shape of the renal anlage and of the earlier stages of its metamorphosis. This is true, not only when considering types with simple non-lobulated kidneys as the cat and rabbit, but also when comparing these with types having lobulated kidneys as man and pig. In each type a renal evagination grows in a dorsal direction into the nephrogenic tissue and in its further growth differentiates into a primary renal pelvis and ureter, the former being enclosed and surrounded by the nephrogenic tissue, which in rabbit and human embryos permits of a differentiation into an inner and outer zone of nephrogenic tissue, as described by Schreiner, while in pig and cat embryos of corresponding stages, a differentiation of nephrogenic tissue into two zones is at this stage not made with certainty. (Compare

Schreiner, pages 121 to 128 and pages 138 to 146.) The further development of the primary renal pelvis and nephrogenic tissue is for cat, pig, and human embryos much the same as above described for the rabbit. For each type, with the appearance of the primary renal branches of the primary renal pelvis, the inner and outer zones of the nephrogenic tissue may be clearly made out, the inner zone breaking up into cell groups which enclose and surround the ends of the branches as these become differentiated. At this stage, the primary renal pelvis for each type studied and modeled presents a middle portion of somewhat irregular shape, the primary renal pelvis proper, from which arises the ureter and an anterior and caudal end, slightly enlarged and more or less lobulated (depending on the stage of development) and three pairs of branches connected with the middle portion; these are not, however, made out with the same degree of clearness and do not present quite the same arrangement in the different types studied. These three pairs of branches are quite readily made out in cat embryos and present an arrangement not unlike that seen in the rabbit (text-figure 21 *B*, Schreiner), while in pig embryos the grouping of the primary branches is not so regular and only after they have attained some size is it possible to make out clearly what may be regarded as three pairs of branches. This, though to a less extent, is true also of human embryos. Attention may, however, here be called to the fact that models of the primary renal pelvis and its branches, in embryos of the same genus and of about the same age, even of the two kidneys of the same embryo (irrespective of size) present slight variation in the form of the primary renal pelvis and in the arrangement of the branches. A difference is also noted in the different types in the shape of the ureter as it enters the primary renal pelvis. In rabbit and cat embryos, this presents only a slight funnel-shaped expansion, while in pig and human embryos the expansion is much greater and is compressed in a dorso-ventral direction with its long axis parallel to that of the renal pelvis. The enlarged anterior and caudal ends of the primary renal pelvis, soon after the anlage of the three pairs of branches to which reference has been made, present, as development proceeds, more and more marked irregularity of form and develop each two, sometimes three, branches, which in appearance are like the other branches, showing a slightly narrowed stalk and enlarged ends; at the same time, the inner zone of the nephrogenic tissue which enclosed and surrounded the enlarged ends of the primary renal pelvis, breaks up into masses which surround only the bulbous ends of the resulting branches. In Fig. I, *A* and *B*, are reproduced two models, obtained by wax reconstruction, of the ureter, renal pelvis and branches of a cat and a human embryo for a stage slightly older

than above discussed, in that the primary branches had already undergone secondary division. In these drawings, the nephrogenic tissue, for the sake of clearness, is not included. The models are purposely shown from different aspects, the one (*A*) giving a lateral view, the other (*B*) a dorsal view with reference to the position of these structures in the respective embryos. In both figures, in *A* more clearly than in *B*, the three pairs of primary branches are still discernible. The bulbous extremities of the primary renal pelvis described for earlier stages show here a division into branches, more clearly seen in *B* than in *A*, the former representing a slightly older stage.

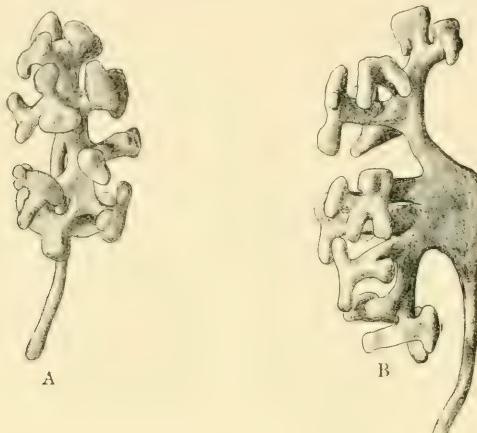


FIG. 1. Wax reconstruction of the ureter, primary renal pelvis, and branches. $\times 50$. *A*, cat embryo (No. 2), 13 mm. in length; *B*, human embryo (No. 5), neck-breech, 16 mm., head-breech, 20 mm. In each figure only about one-third of the length of the ureter is represented.

The primary branches of the renal pelvis, or, as they may hereafter be known, the primary collecting tubules (Nierengangäste—Schreiner), extend to near the periphery of the renal anlage. Each shows, as has been stated, a distal enlargement, which is known as the ampulla (primitive renal vesicle—Haycraft), and which is surrounded by a cap of tissue which we have described as the inner zone of the metanephrogenic tissue. Each ampulla, in its further development, enlarges, the distal wall of the enlargement becoming somewhat flattened and two lateral buds are developed; the primary collecting tubule now presents the appearance of a T or a Y when seen in longitudinal section or in a reconstruction. The distal end of each bud, soon after its beginning, develops a new ampulla. The inner zone of the metanephrogenic tissue which surrounds the primary ampulla as a continuous cell-mass, begins to show a separation into

two parts as the ampulla becomes flattened and the separation becomes complete as the buds or lateral branches develop, each becoming in this way capped by an inner zone of metanephrogenic tissue. The stage here reached is the one shown in the reconstruction reproduced in Fig. 1.

RENAL VESICLES.

In Fig. 2 is shown a portion of a longitudinal section of a developing kidney of a human embryo 18 mm. in length and presenting essentially the same development of primary renal pelvis and branches as that in *B* of Fig. 1; this shows a longitudinal section of a primary collecting tubule, *a*, the distal end of which shows an ampulla, slightly flattened and presenting on each side a lateral extension recognized as the anlage of a branch. The section from which the sketch was made does not pass through the

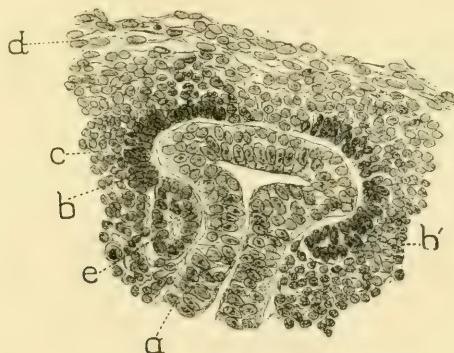


FIG. 2. From a longitudinal section of a developing kidney of human embryo (No. 4), 18 mm. in length. $\times 233$. *A*, primary collecting tubule with ampulla; *b*, *b'*, inner zone of metanephrogenic tissue; *c*, outer zone of metanephrogenic tissue; *d*, anlage of capsule; *e*, renal vesicle.

center of the tubule. It is owing to this that its wall has the appearance of being composed of stratified epithelium. In other sections of this tubule, as also in sections of primary collecting tubules at corresponding stages from cat, rabbit, and pig embryos, it may be seen that their wall is composed of a single layer of cylindrical cells, varying somewhat in length, the nuclei of which are not always in the same plane. In the tissue surrounding the tubule and ampulla here figured, the following may be seen: Immediately surrounding the lateral extensions of the ampulla and extending for a short distance along the tubule there are recognized groups of cells, *b*, *b'*, which may be separated from the surrounding cells. These groups of cells we may know as the inner zone of the metanephrogenic tissue. The cells of this tissue are indistinctly bounded and possess

relatively large oval nuclei which stain rather deeply and have a radial position with reference to the ampulla and tubule. In the preparation figured they are arranged in two rows quite clearly defined. This inner zone of the metanephrogenic tissue presents a sharp demarcation toward the ampulla and tubule, emphasized in the preparation sketched by reason of the fact that the ampulla and tubule were slightly contracted during the process of hardening. Herring has called attention to similar appearances presented by his preparations. In the relatively thin sections into which my entire material was cut, the distinct demarcation between the inner zone of the metanephrogenic tissue and the epithelium of the primary collecting tubules and their ampullar enlargements may nearly always be readily made out, if the plane of the section passes through the lumen of the tubules and their ampullar enlargements, thus giving a cross section to their epithelial walls.

Such appearances as described by Sedgewick, when he states "Some of the larger columnar cells of the kidney tubules become branched, the processes being continuous with the processes of the branched cells of the kidney blastema. In fact, every stage between a columnar lining cell of the tubule and a branched cell of the blastema is visible"—I have not observed. The same may be said of the observations of Riede, who regards the epithelium of the renal evagination and of the primary collecting tubules as contributing to the formation of the renal blastema. It is only in relatively thick sections or in sections which pass obliquely through the epithelial walls of a primary collecting tubule without including the lumen, in which the nephrogenic tissue overlaps the tubular epithelium that a distinction between the two tissues is not readily made. Herring, who speaks of a cap or mantle of cells lying over the end of each ampulla, speaks of a sharp line of demarcation between the tube and the cap or mantle of cells. Ribbert describes groups of cells which surround cap-like the ends of collecting tubules. These groups consist of two or three layers of epithelium-like cells which may be separated from collecting tubules on the one side and from "renal blastema" on the other. Schreiner, for all the types studied by him, describes and figures a distinct demarcation between the inner zone of the metanephrogenic tissue and the primary collecting tubules (*Nierengangäste*).

Immediately surrounding the inner zone of the metanephrogenic tissue there is a tissue in which the cell boundaries are also indistinct, with relatively large, round or oval nuclei, which show no definite arrangement, c., and which do not stain so deeply as the nuclei of the inner zone. This constitutes the outer zone of the metanephrogenic tissue. This zone blends with a tissue which is recognized as mesenchymal tissue. In the mesenchymal tissue, there is recognized a layer in which the majority of the nuclei show an elongated oval form and fairly regular arrangement, their long axis being parallel to the outer boundary of the kidney anlage, entirely surrounding it. In this layer is recognized the anlage of the capsule of the kidney, d. External to it, there is seen a mesenchyme containing relatively few nuclei, not sketched in the figure. The entire renal anlage measures in the embryo from which Fig. 2 was taken almost exactly 1 mm. in length. In the stained sections it is readily recognized with the naked eye as a small area, staining more deeply than the surrounding tissue and having a bean or kidney shape. The appearances presented in sections of the developing kidney of rabbit, cat, and pig

embryos for corresponding stages are in all essentials as here described and figured for the human embryo. In each type the inner zone of the metanephrogenic tissue consists at this stage of generally two, sometimes three, layers of cells, which, as other observers have stated, have the appearance of epithelial cells, always surrounding the ampullæ of the primary collecting tubules or of their branches much as shown in Fig. 2. The same may be said as concerns the structure and disposition of the outer zone of the metanephrogenic tissue and of the capsule anlage.

If the inner zone of the metanephrogenic tissue found surrounding the right ampullar extension as shown in Fig. 2 (*b'*) is traced in sections preceding and following the one shown in the figure, it will be seen that its border is not for its entire circumference an even one, but that it presents a bud-like prolongation which extends for a short distance along the side of the collecting tubule. In this prolongation, which is here cut through its center, the cells show an arrangement in two distinct layers, continuous at the end of the prolongation. I was led to recognize this fact by Schreiner's description of similar stages. Such bud-like prolongations of the inner zone of the metanephrogenic tissue early acquire a narrow lumen, around which the cells assume a radial position, certain cells of the bud in the region of its junction with the main mass of the nephrogenic tissue turning with their inner ends toward the lumen. In this way the bud becomes separated from the main mass of the inner zone. In the figure this process of separation is shown at its very beginning; a lumen is made out with difficulty and one cell, the fourth in the inner row, appears to have been fixed in the act of turning. In the further differentiation of such a cell-mass, the lumen increases in size and the cells surrounding it increase in length, becoming more distinctly columnar in shape. The whole presents now the appearance of a small vesicle, the wall of which is formed by a single layer of columnar cells, which during its differentiation has completely separated from the nephrogenic tissue. Such a vesicle is shown to the left in Fig. 2 (*e*). A number of investigators have observed such vesicles and have interpreted them as representing anlagen of uriniferous tubules. They were first described and quite correctly figured by Emery, who speaks of them as "*vésicules rénales*," later by Riede, very briefly by Hamburger, Chievitz, and Ribbert, and quite accurately by Herring, whose excellent photographic reproductions are worthy of study, and finally by Schreiner, who gives a minute and accurate account of their origin and structure in the different types of animals studied by him. The account here given, based on sections of human embryos, is readily verified in sections of rabbit, cat, and pig embryos of corresponding stages. In each type small vesicles, which we

shall designate as renal vesicles (following Emery, not to be confused with the primitive renal vesicles as described by Haycraft) are differentiated from the inner zone of the metanephrogenic tissue in the manner above described. The first renal vesicles differentiate in the types of embryos studied by me simultaneously with the anlage of the two branches into which the branches (primary collecting tubules) of the primary renal pelvis divide. That the renal vesicles have no connection with the primary collecting tubules may generally be made out by tracing these structures through a series of relatively thin sections; wax reconstructions of the primary collecting tubules and of the renal vesicles give, however, conclusive evidence of their independence.

Investigators who hold that the uriniferous tubules are developed by a process of budding from the primary, secondary and further branches of the epithelial renal evagination do not recognize the renal vesicles as here described. As they can not have escaped their notice entirely, it must be assumed that they are regarded by them as continuous with the developing collecting tubules at all stages of their development and that they were thus interpreted as solid or hollow buds of the collecting tubules. Few of the contributions to which reference is here made contain satisfactory accounts of the earliest stages of the development of the uriniferous tubules nor are they accompanied by figures which clearly portray these stages. The figures presented are, in the great majority of instances, of stages where a direct continuity between collecting tubule and uriniferous tubule can not be questioned. Certain of the figures to which reference is here made showing earlier stages of kidney development deserve brief consideration. We may mention Fig. 581 of Köllicker's *Entwickelungsgeschichte des Menschen und Höheren Thiere* (1879), which shows a longitudinal section of the kidney of an embryo rabbit 16 days old. In this figure are shown several primary collecting tubules ("Endsprossen des Ureters oder Ampullen"), showing T or Y-shaped division of their distal ends and at least seven renal vesicles, if I may be allowed to interpret the figure in the light of observations made on my own sections of rabbit embryos of about corresponding age. In the accompanying text, Köllicker states that by further growth of the branches of the collecting tubules, these form the uriniferous tubules; no mention is made of the renal vesicles. In Figures 1, 2, 3, and 6 (of Plate XXIV) of Löwe's article, sketches of the entire kidney, as seen in longitudinal section of rabbit embryos of 5, 10, 20, and 50 mm. length respectively are shown numerous renal vesicles in various stages of development in close relation with a tissue, which from its form and position must be regarded as the inner zone of the nephrogenic tissue. Löwe, who recognized the cell-masses, has interpreted them as renal vesicles and traced their origin to Braun's cords, stating that from them is developed the endothelium of the capillaries of the Malpighian bodies, while all parts of the uriniferous tubules with the epithelium of Bowman's capsule are derived from the primary ureter branches. Again in Figs. 40 and 41 of Strahl's communication (kidneys of human embryos of 28 mm. and 35 mm. length respectively) the inner and outer zones of the nephrogenic tissue and renal vesicles are recognized. Stoerk's series of figures grouped under Fig. 2, giving in a "semi-schematic" way the development of the ends of the "ureter branches" and the anlage of the Malpighian bodies (*Nieren-körperchen*) show that he has not observed the renal vesicles, but, as No. 4 of this series of figures would indicate, he has interpreted the renal vesicles as buds of the ureter branches, as the position of the downward growing buds, as there figured, coincides with the relations shown by renal vesicles and primary ureter branches at a corresponding stage of development. Mention was made of Stoerk's view of the anlage of the uriniferous tubules on page 5. Toldt, whose observations are frequently quoted as giving conclusive evidence of the development of the uriniferous tubules by a process of budding or further growth of the ureter branches, removed the kidneys from young embryos, stained them in hematoxylin or carmine, cleared them in turpentine and placed them on a slide as a whole. In such preparations, the primary branches of the ureter could be traced without interruption to the periphery of the organ where they terminated in enlargements. A further examination of the ureter branches designated by Toldt as "Zellsprossen" with higher magnification showed that they grew forward first as solid buds which later attained a lumen. In Fig. 1 of the plate accompanying his article (kidney of cat embryo 13 mm. in length) is reproduced such a preparation. This shows however only ureter, primary renal pelvis and its primary branches, surrounded by kidney blastema and gives no evidence of the anlage of the uriniferous tubules. It should be remembered that it is conceded by the majority of observers that the epithelium of the primary renal pelvis and its branches is derived from the renal evagination by growth and budding. The figures given by Toldt and obtained from sections and teased preparations to show the anlage of the uriniferous tubules are of more advanced stages. It must, therefore, be stated that the method selected by Toldt to show the anlage of the uriniferous tubules is not a suitable one. The figures given by Pye, Nagel, Golgi, Minot, Haycraft, Von Ebner, and Disse show that they are dealing with developmental stages that do not represent

the anlage or the beginning of the uriniferous tubules but more advanced stages where union between tubular anlage and collecting tubule had already taken place or that when earlier stages are represented as for instance by Disse (Fig. 77), it seems apparent that a continuity of the discontinuous structures is simulated by an overlapping of the two structures. In sections passing through the epithelium of an ampullar enlargement, without passing through its lumen and through the inner zone of the nephrogenic tissue and a renal vesicle, it often seems as if these structures were continuous, presenting the appearance of a bud growing from the ampullar enlargement of the primary collecting tubule. This is also true if the section passes obliquely through these structures. In such sections, especially if relatively thick, the inner zone of the nephrogenic tissue, which, as has been stated, resembles epithelial tissue, appears as a continuation of the epithelium of the collecting tubule. Haycraft makes the statement that the most conclusive evidence in favor of the view that the epithelium of the kidney tubules arises from the blastema "would be the presence of isolated masses of cells and their subsequent junction with the tubules, but of this, as we have seen, there is no proof." "What we have is an appearance at the tips of the collecting tubules which suggests to the minds of many observers that the cells of the blastema gradually transform themselves into epithelium." He further states that such appearances are due to "nothing more nor less than oblique sections of tubules, not through their extremities, but through bends in their course." To this it may be answered that reconstructions show conclusively the presence of isolated cell-masses—renal vesicles, the anlagen of uriniferous tubules—which subsequently join the collecting tubules and further that the very argument which Haycraft uses to point out the sources of error of those who hold to the discontinuous origin of the uriniferous tubules from special cell masses may be used with equal pertinence to explain the sources of error of observers who hold that the uriniferous tubules are developed by a process of budding from the collecting tubules, namely that the latter have been led to interpret as continuous structures certain discontinuous structures—primary collecting tubules, inner zone of metanephrogenic tissue and renal vesicles—which by reason of their close relation and a certain resemblance in structure of their constituent cells appear in oblique sections as forming a continuous whole. My own observations, pertaining to human, cat, rabbit and pig embryos, as may be apparent from statements here made, have enabled me to confirm the observations of investigators who recognize a discontinuous origin for the uriniferous tubules and who have described small vesicles, variously known as Blüschen, acini, or renal vesicles, as the anlagen of the uriniferous tubules proper.

DEVELOPMENT OF THE COLLECTING TUBULES AND THEIR RELATION TO THE RENAL VESICLES.

The further growth of the kidney is accomplished by a further division of the branches of the primary renal pelvis and their differentiation into the straight collecting tubules, by a constant new formation of renal vesicles and by a differentiation of the renal vesicle into the uriniferous tubules and their union with the collecting tubules. Each primary collecting tubule early divides, as has been stated, into two branches, each of which shows a distal enlargement, an ampulla, capped with a layer of nephrogenic tissue. Each of these branches divides again into two branches, the division beginning with the ampullar enlargement. With the formation of this second series of branches, the zone of nephrogenic tissue which surrounded the ampulla of each primary branch separates into two parts, each of which surrounds the end of one of the resulting branches. Several similar divisions follow in relatively quick succession and with the division of each end branch the nephrogenic tissue which surrounds it separates into parts, which in each instance surround the ends of the resulting branches. The end branches from each division develop ampullar enlargements, which are found at the periphery of the organs under the developing capsule, which enlarges as the kidney develops. (In the developing kidney of human embryos, the end branches are seen to end under the capsule and at the boundaries of the primary

lobules, as will be stated more fully later.) The repeated division of the branches of the primary renal pelvis, as here briefly sketched, results in systems of branched, relatively straight tubules, which extend from the pelvis of the developing kidney to the periphery of the organ and are recognized by authors generally as the anlagen of the straight collecting tubules. Such tubules are lined throughout by epithelium having relatively clear protoplasm and round or oval nuclei, the arrangement of which differs somewhat in the earlier branches from that found in the later branches. In the former, the larger tubules, those nearer the pelvis, the epithelium is for the greater part of embryonic life of a pseudostratified type, while in the smaller tubules, those nearer the periphery, the epithelium is simple columnar. The division of the end branches of these tubular systems continues without much variation from the manner here described to about the time of birth or until the final number of collecting tubules has been formed. Hamburger states that the division of the collecting tubules terminates in the human embryo in the fifth month of foetal life, in the pig in embryos about 14 cm. in length, in the rat and mouse at about birth. The details of the development of the collecting tubules will not be entered upon here, as the necessary data are not yet in my possession. This requires a much more extensive series of reconstructions at different stages of development than I have been able to make, and not for one animal, but for a series of animals, as my limited observations indicate that, while the general plan of development of the straight collecting tubules is the same for different mammals, variations in detail are to be looked for. This may be seen also from Hamburger's account, who briefly considers this question, basing his observations on teased preparations. For the purpose of this account, which is concerned with the development and shape of the uriniferous tubules proper, the brief statement will suffice that the straight collecting tubules are developed by a process of continuous growth and of repeated divisions of the ends of the tubules which grow from the primary renal pelvis. These divisions take place at the periphery of the organ (or of the primary lobes, when present) immediately under the capsule. It has been stated that with each division of the ampullar enlargement of an end branch of a system of developing collecting tubules, the inner zone of the metanephrogenic tissue likewise separates into parts which surround the ends of the resulting branches. During the development of the kidney, this tissue proliferates by mitotic division. At all stages of development it is found surrounding the ampullar enlargements of the end branches of the developing collecting tubules as a layer consisting of one or two rows of cells, possessing oval

nuclei which stain relatively deeply, the layer being in contact with epithelial cells which form the wall of the ampullar enlargement. This nephrogenic tissue is therefore found at the periphery of the developing kidney immediately under the capsule. From this tissue, with each successive division of the ampullar enlargements of the end branches of the collecting tubules, new generations of renal vesicles are differentiated in a manner similar to that previously described for the first generation of renal vesicles. While new renal vesicles are thus differentiating about the ends of the terminal branches of the collecting tubules, those previously formed are developing into uriniferous tubules. Those first formed show the greatest degree of development, the various generations of renal vesicles undergoing essentially the same metamorphosis in developing into uriniferous tubules. Beginning with a relatively early stage in the development of the kidney through the entire period when new tubules are forming, there may therefore be observed a peripheral zone containing the end branches of the collecting tubules showing ampullar enlargements surrounded by nephrogenic tissue, forming and formed renal vesicles, and of these others which show the earlier stages of development leading to the formation of uriniferous tubules. This subcapsular zone stains somewhat more deeply than other portions of the developing kidney and may therefore be recognized with the naked eye. It surrounds the entire kidney except the place where the ureter enters. It has repeatedly been recognized as the zone where the earliest stages of the developing uriniferous tubules are to be found and is described by Hamburger and Stoerk as the neogenic zone ("die neogene Zone"—Hamburger). In this way new generations of uriniferous tubules develop outside of those previously formed, the latter thus coming to lie deeper down in the parenchyma of the kidney and showing according to their age more or less advanced stages of development. The period when the new formation of renal vesicles ceases varies somewhat for different animals. In the rabbit, I have observed their formation as late as the first week after birth, although the number of newly forming renal vesicles is for the later period of embryonic life relatively small. In the cat, newly forming renal vesicles were observed in a kidney removed from an embryo a short time before birth. Herring states that no more tubules are formed in the human kidney after the 8th month of foetal life. Stoerk, who has considered this question quite fully, agrees with Herring. Toldt, on the other hand, states that new Malpighian corpuscles form in dog and man in the entire peripheral part of the kidney for 8 to 10 days after birth. My own human material is too limited to be of any value in determining this question.

DIFFERENTIATION OF THE RENAL VESICLES.

We may now consider the further development of the renal vesicles. As has been stated, these are differentiated from the inner zone of the metanephrogenic tissue and, when completely separated, consist of a single layer of columnar cells with oval nuclei which stain relatively deeply and surround a central lumen. At this stage of their development they are generally quite definitely circumscribed and have a shape which varies from that of an irregularly spherical body to that of an egg. They lie in close relation with the collecting tubules and their ampullar enlargements, these being often slightly flattened in the region of their contact with the vesicles. At least two renal vesicles develop in connection with each ampullar enlargement; these do not, however, present the same degree of differentiation and development, as may be seen from Fig. 2, and is more clearly seen in renal vesicles which develop in connection with the ampullar enlargements of the end branches of collecting tubules which appear after the first and second division of the branches, which arise from the renal pelvis. The branches resulting from each successive division of a collecting tubule form with this soon after their anlage a T-shaped structure; in their peripheral growth, the angle between such branches becomes smaller and they form with the collecting tubule a Y-shaped structure, each branch developing an ampullar enlargement surrounded by nephrogenic tissue. A renal vesicle develops from the nephrogenic tissue on the outside of each branch (therefore nearer the pelvis of the kidney) earlier than from the nephrogenic tissue between the branches. (See also Schreiner, especially text-figure 34.) The renal vesicles, soon after their separation from the nephrogenic tissue, increase in size by active proliferation of their cells by mitotic cell division, the vesicle elongating somewhat and coming in close contact with the ampullar enlargement of the collecting tubules. At the same time it may be observed that the outer wall of the vesicle, that away from the collecting tubule, and especially its upper portion, becomes thicker than the wall of the vesicle in contact with the collecting tubule. This thickening of the outer wall of the vesicle was first described by Schreiner and fully discussed by him. He states, and in this my own observations confirm his account, that the cells of the middle and upper portions of the outer wall of the vesicles elongate, the cells here appearing longer when the vesicles are in sagittal section¹ than in

¹ By a sagittal section of a renal vesicle is here meant a section which cuts a collecting tubule in longitudinal direction and parallel with its lumen and passes at the same time through a renal vesicle which may develop by its side, thus cutting the renal vesicle in a plane which passes through the middle or nearly the middle of its outer wall. The renal vesicles seen in Fig. 2 and 4 of Fig. 3 are cut in sagittal section. The plane of a frontal section of a renal vesicle passes perpendicularly through it and would intersect the plane of a sagittal section at right angles.

other parts of the vesicles and that evidences of cell division are often seen.

In such a section it will be seen that the lower inner ends of these elongated cells, which no longer hold a radial position to the lumen of the vesicle, but are inclined to its long axis, slightly overlap the inner ends of the cells which form the lower portion of the outer wall of the vesicle, so that a portion of the outer wall of the vesicle presents the appearance of a two-layered epithelium. As development proceeds, the outer wall of the vesicle further thickens and encroaches on its lumen, which in sagittal sections now presents the appearance of a hook-shaped space. At about this time there appears on the outer wall of the vesicle over its thickest portion a slight depression, beneath which a cleft makes its appearance; this begins to separate the thickest portion of the wall of the vesicle into two layers. This cleft deepens as the two layers of cells become more distinctly separated. In *A* of Fig. 3 is shown a sagittal section of a renal vesicle at this stage of development. The section sketched does not pass exactly through the center of the vesicle, nor is it quite parallel to its long axis. This needs to be remembered in examining this figure, as the arrangement and size of the cells forming the different parts of the wall of the vesicle are not quite what might be expected from the foregoing description. The reproduction gives the most typic section of the series of sagittal sections of the vesicle from which the reconstruction was made which is shown in *A* of Fig. 4. In *A* of Fig. 3 is shown an ampullar enlargement of a terminal branch of a collecting tubule surrounded by nephrogenic tissue, a renal vesicle and the surrounding mesenchyme and a portion of the capsule. In the wall of the vesicle away from the collecting tubule may be observed a slight depression and beneath this a cleft. The nuclei of the cells adjacent to the cleft are stained a little more deeply than the other nuclei of the vesicle. Such an appearance is now and then seen, though not characteristic of this stage. In the reconstruction, this cleft appears on the surface as a narrow depression placed nearly transversely to the long axis of the vesicle, a little above its center, though not extending across its entire wall, and is therefore scarcely seen in the exposure of the model as sketched. That portion of the vesicle, which in sagittal section shows the narrow cleft, is in reality a small pocket, the upper and lower walls of which are nearly in contact at this stage of the development. Similar appearances I have observed in a number of other wax reconstructions of this stage. This pocket is not developed primarily by an infolding or invagination of the outer wall of the vesicle, but differentiates in a thickening of its outer wall. This thickening

results from a slight readjustment of the cells constituting this part of the vesicle as well as by their proliferation. In the further development, this cleft enlarges by deepening and by extending laterally so as to involve more than the outer wall of the vesicle. In this way the lower and outer part of the original vesicle becomes separated from the main part of the vesicle and presents the appearance of a lip, which projects upward toward the periphery of the kidney. Into this lip-shaped portion the lumen of the vesicle extends. While these changes in form and structure which affect primarily the lower part of the vesicle are taking place, the upper part of the vesicle elongates, sometimes more and sometimes less, growing toward the respective collecting tubule, more correctly its ampulla; at the same time, a slight depression appears in the uppermost part of the inner wall of the vesicle, that in apposition with the wall of the collecting tubule, which emphasizes the curvature of the upper part of the vesicle toward the collecting tubule. In *A* of Fig. 3 is shown the first indication of this second depression in the wall of the renal vesicle at a point marked by a cross. The end result of these changes in form and structure on the part of the renal vesicle are represented in *B* of Figs. 3 and 4. It may be seen that by a deepening of these clefts, the more prominent one in the outer wall, the less prominent one in the upper portion of the inner wall of the original vesicle, and by a growing of its upper end toward the collecting tubule, the vesicle has been altered in its shape so as to present in sagittal sections and at times also in reconstructions the form of an S. In the sagittal section of this renal vesicle, or, as we shall now know it, the tubular anlage (the word uriniferous being understood) is cut longitudinally and through its middle in the section sketched for nearly its entire length, the upper portion of the anlage deviating a very little from this plane of section. The reconstruction of this tubular anlage, *B* of Fig. 4, does not show this S-shape as clearly as certain of the sections of it. The upper bend of a tubular anlage of about this stage presents a circular and very narrow lumen. In the tubular anlage figured, the lumen of its upper bend appears clearly only in the section sketched. In frontal sections of tubular anlagen of this stage, in which this upper bend would appear in cross or slightly oblique section, the existence of a small lumen in this portion of the anlage may be clearly made out, the part itself being round or nearly so. It constitutes, therefore, a tubular structure, its wall being made up of a single layer of columnar cells with oval nuclei. Frontal sections of a tubular anlage of this stage further show that its lower bend does not represent a structure having a tubular form, but is flattened from above downward, presenting in such sections the

appearance of a crescent with concavity directed upwards toward the periphery of the kidney. Reconstructions of tubular anlagen of this stage show that this portion presents the shape of the bowl of a spoon or of a saucer with the concavity turned toward the other parts of the anlage. In making this comparison, it is to be understood that this structure is not solid, but double-walled, the enclosed space, which is continuous with the lumen of the other parts of the anlage, having a similar shape. At this stage in the development, the upper concave wall of the structure is composed of columnar cells, while the lower or convex wall is composed of cubical cells. Both in the series of sections of the tubular anlage shown in *B* of Fig. 3, and also in the reconstruction of the same, *B*, Fig. 4, it may be clearly seen that it is not continuous with the ampullar enlargement of the collecting tubule. It is in close contact with it but not continuous with it. In *C* of Figs. 3 and 4 is shown a tubular anlage which is only slightly further advanced in its development than the one shown in *B* of the same figures. The section sketched shows, however, a continuity of the lumen of the tubular anlage and that of the ampulla of the collecting tubule, although, as is evident from the sketch, the place of union of the two previously discontinuous parts may yet be made out, partly by reason of the position of the nuclei of the respective cells, also because there is as yet a slight constriction at the place of fusion, this being not as yet complete. The *modus operandi* of this union between tubular anlagen and collecting tubules has been discussed by Schreiner, who states that he has observed in the tubular anlagen or in the ampullar enlargement of the collecting tubule at a stage which he has recognized as just previous to a stage in which there is union of the two, a cell in mitotic division or cells which by their structure and staining show that they have just completed division. This division of cells at the place of contact of the upper end of the tubular anlage and the ampulla of the collecting tubule, Schreiner associates with the fusion of these parts and with the formation of a continuous lumen. This stage is rather difficult to find, as it is not readily recognized unless the respective tubular anlage is seen in a favorable sagittal section. In the tubular anlage, a section of which is here shown, there is no evidence which would indicate that one or more of the cells situated at the place of its junction with the collecting tubule had just completed its division. In my own preparations I have not found cell division at the place of contact between tubular anlagen and collecting tubules more frequent than in other parts of these structures in a stage which might be interpreted as just prior to a fusion of these structures. My own observations would, therefore, confirm only in part those of Schreiner as con-

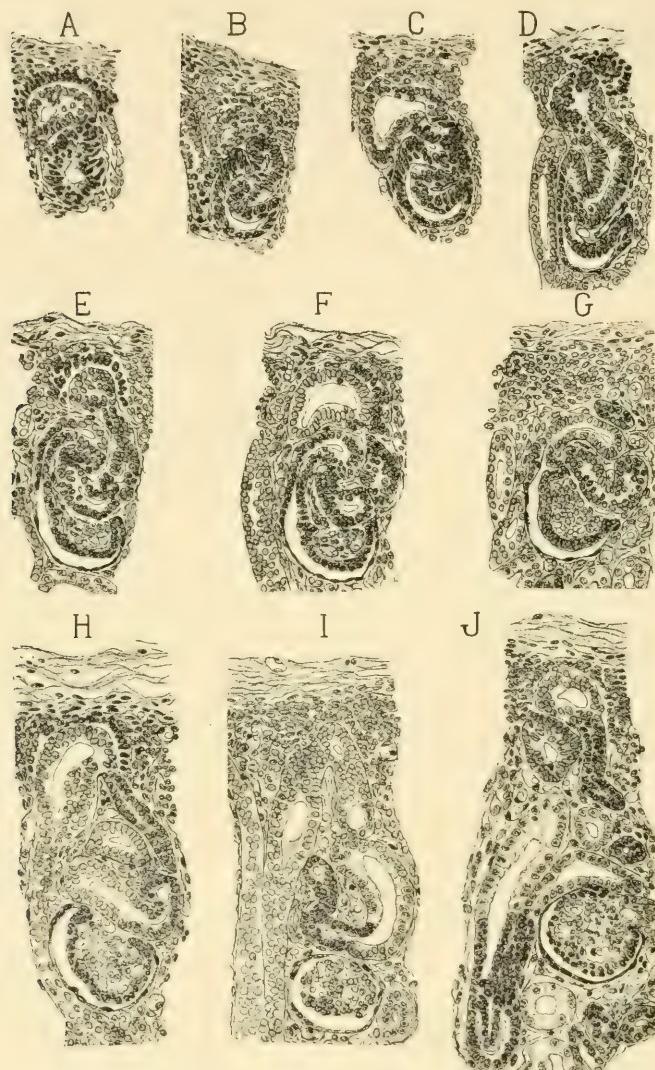


FIG. 3. A series of figures, *A* to *J* of sagittal sections of tubular anlagen and uriniferous tubules in early stages of development, showing successive stages in the development of the uriniferous tubules, from a human embryo of the seventh month (No. 16). $\times 160$. In each figure of this series, there is reproduced the most typical section of the several series of sagittal sections used in making the reconstructions shown in Fig. 4. (The corresponding tubules in Figs. 3 and 4, are designated by the same letter.)

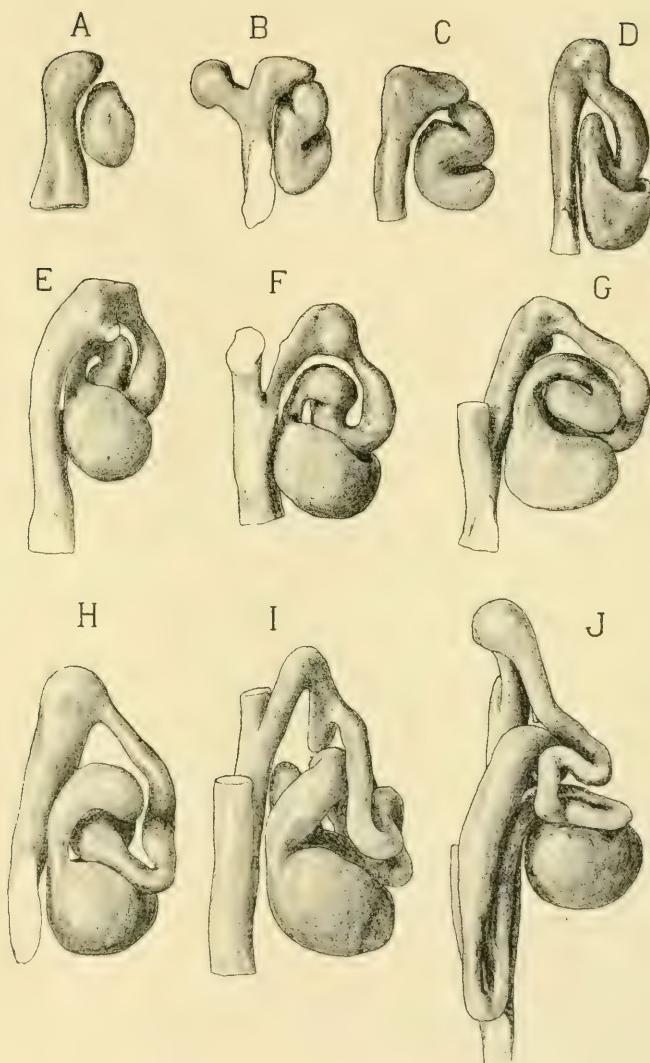


FIG. 4. A series of models *A* to *J*, showing successive stages in development of tubular anlagen and early stages in the development of uriniferous tubules, with a portion of the collecting tubule to which each is attached; from a human embryo of the seventh month (No. 16). $\times 160$.

cerns this point. I have, however, accepted Schreiner's account of the processes of union of the tubular anlagen and collecting tubules as the most satisfactory that can be given. In Fig. 5 are reproduced a series of wax reconstructions of the anlage and early developmental stages of uriniferous tubules obtained from rabbit embryos; in Fig. 6, a similar series obtained from cat embryos, and in Figs. 9 and 10, a primary collecting tubule, with its branches, the renal vesicles, tubular anlagen, and uriniferous tubules in early stages of development associated with them. These were obtained from kidneys of human embryos of 3 cm. and 6.5 cm. length respectively. In *A* and *B* of Fig. 5 are represented in each instance a collecting tubule with prominent ampullar enlargement and a renal vesicle. In *A* the renal vesicle is of spherical shape, in *B* of egg shape, the latter representing a slightly older stage, showing, however, as yet no characteristic differentiation; both are distinctly separated from the respective collecting tubule. In *C* of Fig. 5 is reproduced a tubular anlage, which had united with the collecting tubule at a relatively early stage, before presenting the S-shape to which attention has been called. The lumen of this tubular anlage, as seen in sections of it, shows this shape more clearly than does the reconstruction. The same may be said of the tubular anlage shown in *A* of Fig. 6, the earlier stages of the series being here not reproduced. These two figures have been introduced to show that the tubular anlagen do not always present the same degree of development and differentiation at the time of their union with the collecting tubules. Characteristic and constant differences in this respect I have not observed for the different animals studied.

Variations in the time of fusion of the tubular anlagen and the collecting tubules within the limits shown in these figures may be observed in a series of sections from developing kidneys obtained from embryos of the same species of animals. Such fusion does not take place, so far as my observations go, prior to a time when the renal vesicles show a distinct indentation of their outer wall, the lumen thus presenting the shape of a hook, but may take place before the inner upper portion of the vesicles or tubular anlagen show an indentation leading to the formation of the upper curvature; in this case the curvature develops after fusion has taken place. A study of the tubular anlagen shown in Fig. 9 and a comparison of these with those shown in Figs. 4, 5, and 6, will prove instructive, especially the two tubular anlagen seen to the left of the upper ends of the two prominent diverging branches of the collecting tubule. Since the latter are sketched from a different view than those of the previous figures, they represent tubular anlagen just after fusion

with the collecting tubules and show clearly the saucer-shaped expansion of the lower bend of the S-shaped structure characteristic of this stage.

S-SHAPED STAGE IN THE DEVELOPMENT OF THE URINIFEROUS TUBULES.

To characterize the S-stage of the development of the uriniferous tubules more clearly, one may speak for convenience of description of an upper S-curve, with concavity toward the collecting tubule; an S-middle piece or middle S-segment, and a lower S-curve with convexity toward the collecting tubule. In considering the tubular anlage as a whole, one may speak of its inner face or side, that turned toward the collecting tubule, and an outer side, that turned away from the collecting tubule and further of a front and back side or face, these with reference to a plane passing through the middle of a tubular anlage as in a sagittal section of the same, and finally of an upper and lower portion of the anlage, with reference to its attachment to the collecting tubule, which would mark its upper portion. In the same way we may speak of a portion of a tubular anlage as curving or growing inwards or outwards, upwards or downwards, and forward or backward. In the further description I shall make use of these terms without further comment. In this connection it needs to be recalled that renal vesicles and tubular anlagen develop in connection with each of the two end branches resulting from the successive divisions which occur in the course of the development of a collecting tubule. Favorable sections now and again show a collecting tubule with two more or less clearly differentiated end branches connected with two tubular anlagen seen in sagittal section. The whole structure presents the appearance of a Y or of an anchor with the arms bent toward the stem of the Y or shaft of the anchor (see Figs. 10 and 12). Assuming that the two tubular anlagen are in the S-stage, only the left one would in reality have the shape of a Roman S, while the right one would show a mirror picture of the same. This fact enables one readily to state whether any particular tubular anlage is placed to the right or left of a given collecting tubule. By right or left is meant the relations shown by these structures to the collecting tubule to which they are attached in any given section. In the series of figures showing reconstructions of tubular anlagen and early developmental stages of uriniferous tubules, as given in Figs. 3, 4, 5, and 6, I have for each stage of development selected a tubular anlage placed to the right of a collecting tubule. It seemed to me that this would assist materially in tracing the successive stages and would facilitate a comparison of similar stages. Tubular anlagen placed to the left of the collecting tubules show the same developmental stages which would appear in

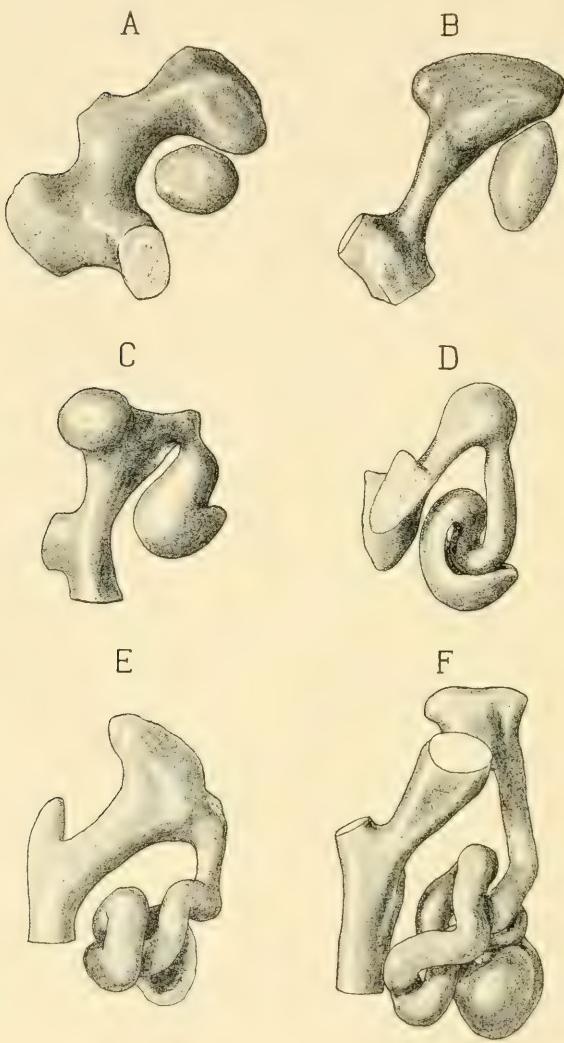


FIG. 5. A series of models *A* to *F*, showing successive stages in the development of uriniferous tubules in rabbit embryos. $\times 160$. *A*, *E*, and *F*, from rabbit embryo (No. 2) of 14 mm. length; *B*, *C*, and *D* from rabbit embryo (No. 3) of 18 mm. length. *A* and *B* show each a renal vesicle and a part of a collecting tubule with prominent ampulla; *C* and *D* early and late S-shaped tubule.

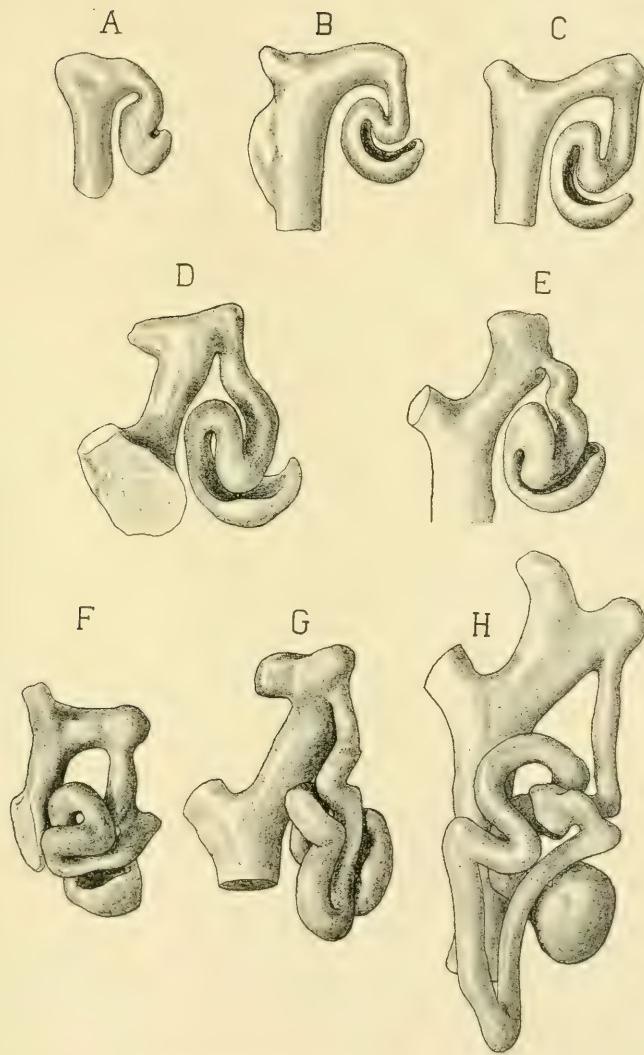


FIG. 6. A series of models, *A* to *H*, showing successive stages in the development of uriniferous tubules in a cat embryo 22 mm. in length. $\times 160$.

mirror pictures to those here given and show the same relations to the collecting tubules. They show similar inner and outer, front and back surfaces as above described, these designations having reference to the relations of any tubular anlage to the collecting tubule to which it is attached, independent of the fact that such anlage may be placed to the right or left of the collecting tubule. With this definition of the terminology to be used, we may now proceed to a fuller consideration of the S-shaped stage and state that the upper S-curve represents a tubule with narrow lumen having an outward curvature and extends without definite boundary into the S-middle piece, which is also of cylindrical shape, representing a short tubular segment also with narrow lumen and, like the upper S-curve, is composed of a single layer of cells with oval nuclei. When first recognized, it has a nearly horizontal position; but as development proceeds and the S-shape of the tubular anlage becomes more pronounced, it loses this horizontal position and inclines upward toward the collecting tubule. This portion of the anlage is not so clearly defined as the other parts and the relative time of its differentiation varies somewhat, as will be more fully stated later. The lower S-curve, as has been stated, is not of cylindrical shape, but from the time of its anlage is flattened from above downward and presents the form of a double-walled saucer or shallow bowl with concavity directed upward, the cavity which extends into this portion being likewise flattened and presenting a similar shape. The cells forming the upper wall of this structure are of columnar shape, those forming the lower wall of cubical shape or even more flattened, depending on the stage of development. The saucer-shaped structure becomes narrowed as the region where it joins the S-middle piece is approached and here gradually changes (in the most typical anlagen) from a flattened to a nearly cylindrical structure. The place of junction of the two parts is indicated by a sharp bend in the lumen of the tubular anlage as this is seen in sagittal sections and may be indicated by a nearly equally sharp bend when the anlage is seen in reconstructions. The lower end of the upper S-curve, with a portion of the S-middle piece, rests in the concavity of the saucer-shaped portion representing the lower S-curve so that only a narrow space separates these parts. In sagittal sections this space appears as a curved cleft and contains, soon after its anlage, a delicate strand of mesenchymal tissue. (See *C* of Fig. 3.) In *D* of Figs. 3 and 4 is shown a slightly older tubular anlage, which differs from the former in that the place of union between the tubular anlage and the collecting tubule is no longer recognized and in the more pronounced curvature of the anlage as a whole. This is especially clearly seen in the section

of this tubular anlage shown in *D* of Fig. 3. In the reconstruction it will be seen that what appears in section as the lower S-curve represents in reality a shallow bowl, the edges of the saucer-shaped structure recognized in the former stage and figure having grown upward, thus deepening the concavity; also the space which separates this part of the anlage from the lower end of the upper S-curve and S-middle piece has increased somewhat and is now occupied by a vascularized mesenchyme, the section reproduced showing two capillaries, the one cut cross-wise, the other seen in longitudinal section. The mesenchyme contained within the concavity of the lower S-curve has long been recognized as the anlage of a glomerulus, this portion of the tubular anlage, as may be stated now, forming Bowman's capsule. My own observations lead me to think that the first capillary loop found within the mesenchyme occupying the concavity of the lower S-curve of a tubular anlage grows into this from without, as I have generally been able to trace a connection between it and the capillaries outside of the cleft occupied by the mesenchyme. To give definite answer to this question, however, it would be necessary to confirm the above statement on injected preparations, but the fresh material of the proper stage of development, obtained during the course of this investigation, was too limited to attempt this. This question has received only incidental mention by other investigators; of the more recent of these we may mention Schreiner, who simply states that "into the cleft there grow from without connective tissue and vessels," and Stoerk, who, in speaking of the development of the S-shaped stage of the tubular anlage, states that "there is formed between the upper curve and the middle piece on the one side and the concavity of the lower curve on the other side a downward curving cleft, which takes up vascularized interstitial tissue, from which later the glomerulus is developed." Herring, on the other hand, states that when the S-shaped tubule is formed, the space between the lower and the middle limb is occupied by some connective tissue cells; they are few in number and resemble the other cells which are found in the matrix of the cortex. He further states that "From these cells I believe the capillaries of the glomerulus are formed *in situ* and not as an ingrowth."

The S-shaped stage in the development of the uriniferous tubule, especially characteristic when seen in sagittal sections, has long been recognized and was first correctly described by Toldt and is clearly brought out in Golgi's semidiagrammatic figures of developing uriniferous tubules of mammals. The series of developmental stages leading to the formation of the S-shaped tubular anlage have, I believe, been correctly described only by Schreiner, whose account my own observations, as given here, in the main confirmed.

Ribbert gives in a series of semidiagrammatic figures (Fig. 3 of his article) his view of the development of the S-shaped stage of the tubular anlagen. By means of these figures, he aims to show that the lower portion of the cell-mass from which a tubule is developed, in its growth turns sharply upward, toward the periphery of the kidney. In this way, a cleft is formed between the elongation and the cell-mass from which it develops; the latter now makes union with the collecting tubule, the

whole structure acquiring a lumen and the S-shaped stage of the tubular anlage is reached. Herring states that "By active proliferation of cells, the vesicle grows in length, but not as a straight tube; it becomes comma-shaped, the bend of the comma being always away from the collecting tubule with which it eventually joins." "The comma is now in close contact with the ampulla, but the lumens are not continuous. As the comma-shaped body grows, it elongates, but the tail remains in the same position; further growth is upward toward the capsule along the end of the collecting tube. It grows more quickly than the latter and takes on another curve at its upper end, this time toward the ampulla. The result is an S-shaped body, with central lumen. When the S is well developed, its lumen becomes continuous with the lumen of the collecting tube." In both of these accounts, the anlage of the lower curve of the future S-shaped structure, as above given, is missed. Hamburger states that when the anlage of the coiled uriniferous tubule has reached a diameter of 40μ to 50μ , a small cavity develops in its center (renal vesicle) and shortly after this, a union between it and the ampulla is established and a short solid cord of cells, which extends from the peripheral end of the ampulla to the lateral wall of the vesicle develops. In its further development, the tubular anlage obtains a depression, always seen on the side of the anlage which is turned away from the ampulla with which it is connected; its wall sinks in somewhat, thus giving the cavity the form of a "half-moon shaped cleft;" this depression deepens and develops into a cleft and there is formed a "wing-like process," which lies against the side of the anlage and terminates with a sharp free border, which projects toward the surface of the kidney. When the anlage of the coiled uriniferous tubule has attained the size of 50μ to 60μ it still has a spheric shape" but consists of 1, a bowl (Schale), the concavity of which is turned toward the surface of the kidney, 2, a short thick tubule of S-form, which unites this bowl with the ampulla." This stage of the development corresponds with the S-shaped stage of other authors. Such early fusion of the anlagen of uriniferous tubules with collecting tubules as described by Hamburger I have not observed. This error of observation on his part (for such I take it to be) is, I believe, accounted for by the thickness of the sections which he examined (15 to 20 μ). In sections of this thickness, it is often exceedingly difficult to state whether or not renal vesicles or tubular anlagen are merely in contact with the collecting tubules or continuous with them, unless the parts are most favorably sectioned, a point to which I have previously called attention. That the depression on the outer side of the renal vesicle and especially the cleft which opens into it is not primarily formed by an infolding of the wall was brought out in describing its formation; the solid cord of cells uniting the ampulla with the renal vesicle, as described by Hamburger I have not observed. The upper portion of the renal vesicle retains a lumen as it elongates toward the collecting tubule and this becomes continuous with that of the collecting tubule at the time when fusion between these structures takes place.

Authors who regard the uriniferous tubules as developed directly from the collecting tubules are quite unanimous in their accounts of the formation of this S-shaped stage of the uriniferous tubules, stating that the end branches of the uriniferous tubules, or buds from these, grow for a short distance toward the pelvis of the kidney and then again toward the periphery, the lobule or bud thus becoming flexed, presenting first a concavity, then a convexity toward the collecting tubule. I have previously quoted Haycraft, Gerhardt, and Stoerk as concerns this point. Minot considered the capsule as playing an important rôle in the formation of the tubular anlagen. He states: "The capsule seems to prevent the further elongation of the branch in its line of growth, and to force the end of the branch to curl over, thus by simple mechanical condition causing the formation of the anlage of the Malpighian corpuscle." Gerhardt also states that the capsule offers a mechanical obstacle to the further growth of the straight tubules and thus gives the first impulse to the coil formation. Assuming for the moment that the uriniferous tubules are developed by a growth and budding of the collecting tubules, it must be evident that other factors than the one found in the resistance offered by the capsule to the further elongation of the branches of the collecting tubules, play a part in causing these branches to curl

over and form the anlage for the uriniferous tubules, as in the developing kidneys of human embryos of from 3 cm. to 6 cm. in length the neogenic zone is found, not only at the periphery of the organ, immediately under the capsule, but forms septa which extend nearly to the pelvis of the kidney and are found at the line of union of 4 to 6 primary lobules into which the parenchyma of the organ may at this early stage of its development be separated. These septa, which Haugh has termed "the primary columnæ Bertini," are in reality composed of the neogenic zones, separated at the time when they are first recognizable and for some little time after by only minimal traces of mesenchymal tissue, which occurs here and there in small areas and does not present at this stage of development the relatively dense structure of the capsule, which dips down at the superficial boundaries of these primary lobules for a variable though always short distance. Uriniferous tubules develop on each side of these septa in the same way as under the capsule and it is evident from a study of sections that the end branches which reach these septa or primary columnæ Bertini do not meet resistance in their further growth by the capsule or by a tissue of like density, since such tissue is not found in the primary columnæ Bertini, when first formed and for some little time after.

In Fig. 7 is reproduced a portion of a section from a series of longitudinal sections of a kidney obtained from a human embryo measuring 6.5 cm. in length (No. 11), showing the junction of two primary lobules. In this figure is represented a primary columnæ Bertini, which extends from the capsule nearly to the pelvis of the kidney and the renal parenchyma developing on either side of it. As will be observed, collecting tubules grow toward the septum from either side and renal vesicles and tubular anlagen develop on either side of it in a manner similar to those which develop under the capsule. For the greater part of its extent this columnæ or septum contains at the stage of development shown in the figure very little mesenchymal tissue. This is well developed only near the pelvis of the kidney, from which it extends outward for a short distance and here clearly separates the two lobules. For a fuller consideration of the primary lobulation of the developing kidney and of the primary columnæ Bertini, the reader is referred to Haugh's paper, who considers these questions at some length.

Stoerk, in his second paper, discusses and figures (Figs. 3-8) wax reconstructions of tubular anlagen showing the S-shape. I desire to consider somewhat more fully his observations relating to this stage of the development of the uriniferous tubules, as these observations are not in harmony in all particulars with my own. Stoerk in this paper begins his description of the development of the uriniferous tubules with a stage, which, in profile view, presents the appearance of an inverted question mark. This twisted structure simulates, as he states, in sections the appearance of an S-shaped tubule and is so recognized by authors. With reservation, he adopts the same designation. In this he recognizes, for convenience of description, 3 parts (and in

this I have followed him), an upper S-curve, an S-middle piece and a lower S-curve. The upper S-curve passes without definite boundary into the S-middle piece. The lower S-curve grows upward toward the lateral bend of the upper S-curve; in this way, there is formed between it and the S-middle piece on the one hand and the concavity of the lower S-curve on the other hand, a curved cleft, into which vascularized mesenchyme grows and forms the anlage of the glomerulus. From his reconstructions, he was able to see that the lower S-curve is in reality not a tubule, but is spread

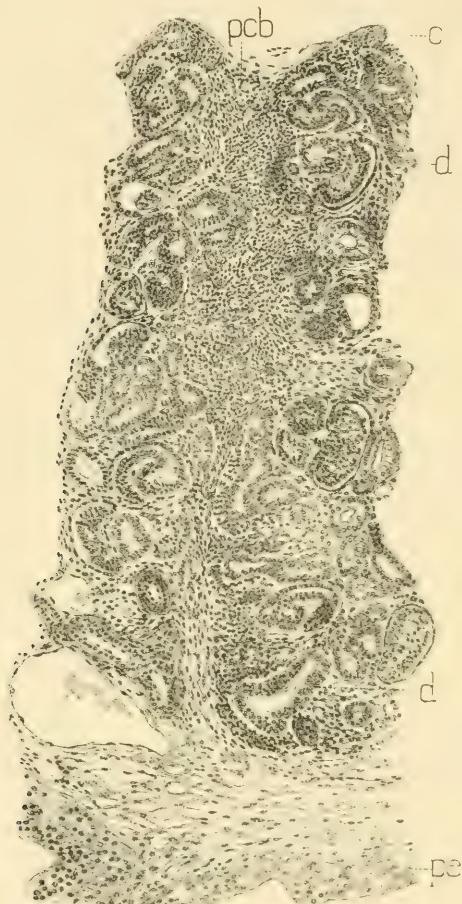


FIG. 7. A portion of a longitudinal section of the kidney of a human embryo measuring 6.5 cm. in length (No. 11), showing a primary columna Bertini, which extends from the capsule to near the pelvis of the kidney. $\times 80$. *Pcb*, primary columna Bertini; *c*, capsule; *pe*, epithelium of pelvis; *d*, end branches of collecting tubules.

out, beginning with the place where the S-middle piece passes into it "into a thin, spherically curved and downward bellying structure with a sharp border, having the form of a triangle with rounded angles; the base of this triangle represents the up-curved, blind ending of the S-shaped tubule, as seen in sections. Over the middle of this structure with upward facing concavity is found just a little above it the junction of the upper S-curve with the S-middle piece." The lower curve of the S, which is not solid but hollow has therefore the shape of an oyster shell ("Muschelschale") and, to continue the comparison, we may say with one having a deeper concavity with edges rolled in somewhat, the more fully developed the structure is.

The account of the further development of the lower S-curve as given by Stoerk I shall give in his own words, as it is particularly with this portion that I desire to take exception. He states (page 297): "Die Muschel nimmt einseitig im Sinne der Fläche an Grösse zu, andererseits gewinnt sie an Wölbung, sodass sie zunächst der Form einer ein wenig verzerrten halben Kugelschale zustrebt (*s. Fig. 3, 4 und 5*); diese unwölbt die Uebergangsstelle vom Mittelstück in die obere S-Hilfste und wird von dieser Kanälchenpartie fast bis zum Kontakt erfüllt; allmählich wird dann auch das S-Mittelstück zur Kugelschalenbildung herangezogen und geht in diese auf (*Modell B*), so dass nunmehr der obere S-Bogen übrig bleibt, der, aus der Kugelschale aufsteigende, von deren Innenseite durch eben jenen schmalen von Zwischengewebe erfüllten Raum getrennt wird, der sich, wie erwähnt, im Schnittbilde als abwärts gekrümmter enger Spalt präsentiert."

This double-walled structure, developing from the lower S-curve and the S-middle piece, with the enclosed mesenchyme, develops into Bowman's capsule and glomerulus. While these are developing, and very soon after the stage is reached to which reference is made in the quotation, the upper S-curve elongates and becomes flexed and for a time again has the shape of an S. This Stoerk designates "secondary S," in contradistinction to the primary S, of which, after Bowman's capsule is formed, there remains only the upper S-curve. In reality, as he states, there is no primary S, this being seen only in sections of this structure at this stage. He further states that the want of recognition of these two S-stages and their time relation, on the part of former observers, accounts for the contradiction which is found between his view and the views expressed by other observers as to the genesis of the different parts of the uriniferous tubules, with reference to the different parts of the S-figure. In discussing these observations of Stoerk, I desire in the first place to call attention to the fact that, while the various anlagen of uriniferous tubules from a time when they may be differentiated from the renal vesicles to a time when they present a distinct S-shape show at different stages of their development certain typic forms which are again and again met with, they after all vary in certain details both as to size and form and also as to the relative time at which certain changes in form occur. This is true not only when comparing tubular anlagen obtained from different species of animals, but also when comparing such obtained from embryos of the same species. With some reservation, the statement may be made that tubular anlagen which develop from the first few generations of renal vesicles are larger and present a slightly different form from those which develop from later generations. In the earlier stages of the development of the kidney, the branched collecting tubules are relatively far apart and the renal vesicles and tubular anlagen which develop in connection with any one system of end-branches are separated by a relatively large amount of interstitial tissue. This enables the tubular anlagen which develop at this period, when these are considered as a whole, to assume a more rounded shape than can the tubular anlagen which develop in the neogenic and subneogenic zones in the later periods of kidney development at a time when the tubular struc-

tures are more crowded and separated by a relatively small amount of interstitial tissue; consequently the tubular anlagen which develop at this period, when they are considered as a whole, do not present a rounded form, but a form which is elongated from above downward. This may be seen when comparing *C* and *D* of Fig. 4, and *B* and *C* of Fig. 6, with the two anlagen shown to the left of the two prominent branches as shown in Fig. 9, and no doubt explains, in part at least, the difference in form of early stages of tubular anlagen as given in Stoerk's figures and the majority of those here represented. I am led to assume, both from a study of his figures and from the statements which he makes, that it is advisable to study the anlage and early developmental stages of the uriniferous tubules in as young embryos as possible, because the elements of the neogenic zone are here farther apart; that his reconstructions are made from very young embryos, while my own were made from relatively older stages—the series shown in Fig. 4 from a human embryo of the seventh month. Besides such differences in shape as here indicated, there are observed in tubular anlagen which show essentially the same stage of development, especially when these are seen in sagittal section, minor differences in shape involving the extent of the curvature of different parts and the relative size and shape and degree of differentiation of the lower S-curve, the saucer-shaped expansion of the S-shaped tubular anlage, when this is compared with its other parts. Of the series of models in my possession showing the S-stage of development of the uriniferous tubules, no matter whether we speak of those made from different species or of those from the same species, no two are exactly alike or even nearly so. They all show certain characteristics of form which enable a classification as to stage of development, but differ when compared in detail. This is more particularly true as concerns the form and size of the lower S-curve and its relation to the S-middle piece. The lower S-curve is developed primarily, as will be remembered, not by an invagination of the outer wall of the renal vesicle, but by a cleft which develops in a thickening of the outer wall. The extent of this cleft varies. It may not, as has been stated, extend, when first recognizable, on to the front and back surfaces of the renal vesicle and may be quite deep before the lower part of the vesicle, that part which will develop into the lower S-curve, has by extension of the cleft to any appreciable extent been separated from the part of the renal vesicle just above it, the part which will form the S-middle piece. Such a structure in reconstruction does not present the form of an S, but may do so in sagittal sections. On the other hand the cleft which appears in the outer wall of the vesicle may, almost from its beginning, extend for

a distance on its front and back surfaces, in which case the lower S-curve and S-middle piece would be recognized in a reconstruction about as soon as in sections. In both cases there develops from the renal vesicle, by a deepening of the cleft and by a growth of the structure, a part which we have known as the S-middle piece and a portion known as the lower S-curve. The former develops as a short tubular segment of irregular cylindrical shape, the latter as a double-walled saucer-shaped structure, with concavity upwards and presenting, as seen from above or below, either an oval or more rounded or irregular triangular shape, with a distinct border at which the two layers of the structure are continuous. In the former case these parts are differentiated at a relatively later period than in the latter, when the form of the anlage is compared with its internal structure, as seen in sections. Before the S-middle piece is completely differentiated, the border or edge of the lower saucer or shell-shaped structure extends for a variable distance on to the S-middle piece and recedes gradually from this as it becomes more clearly defined. So far as my observations go, from a time when the S-middle piece may be considered as fairly well defined, it can be traced as such into the older developmental stages and is not taken up into the lower S-curve as this changes from a saucer-shaped structure to one having a deeper concavity and presenting the shape of an irregular hemisphere, as is stated by Stoerk. This appears to be the case in tubular anlagen in which the border of the lower S-curve or saucer-shaped structure extends for an appreciable distance on to the S-middle piece, but this I interpret as due to a relatively late differentiation of the S-middle piece and not as showing that it is being taken up into the lower S-curve as in tubular anlagen, where the S-middle piece differentiates relatively early, this appears to form a definite tubular segment, which is not lost in its further development. As a matter of fact, not all of the lower S-curve, as seen for instance in a tubular anlage such as is shown in *D* of Figs. 3 and 4, differentiates into the double-walled concavo-convex structure, which forms, in its further development, a Bowman's capsule; but this point shall be discussed more fully in presenting the further stages in the development. I see, therefore, no reasons for recognizing a primary S and a secondary S, as is done by Stoerk, my observations, as stated, leading me to think that the different parts of a tubular anlage which would correspond to his primary S-stage, so far as differentiated, continue as such into the stage which he has designated as the secondary S-stage, the various parts—upper S-curve, S-middle piece, and lower S-curve—being in the latter more clearly defined than in the former; especially is this true of the S-middle

piece, which becomes more clearly defined and does not disappear in the transition from the primary to the secondary S-stage, and this supposed disappearance of the S-middle piece, as the one stage passes into the other, appears to me as the crucial point in Stoerk's argument for the recognition of these two S-stages. In making these statements, I am more especially guided by observations made on reconstructions of tubular anlagen, obtained from developing kidneys of cat embryos varying between 20 mm. and 30 mm. in length and from a human embryo of the seventh month rather than on those made of tubular anlagen developed from the first few generations of renal vesicles of relatively young human and rabbit embryos, as in the former, more generally than in the latter, the S-middle piece differentiates relatively early and consequently such tubular anlagen show at a relatively early stage an S-shape when seen in reconstruction.

Before leaving the consideration of the S-shaped stage in the development of the uriniferous tubule, brief mention may yet be made of a term which was introduced by Colberg and which has now and again been used to designate this stage. Colberg, in an article in which he attempts to controvert some statements made by Henle concerning the shape and structure of uriniferous tubules, which need not be entered upon here, gives also some observations made on the kidneys of a human embryo of the seventh or eighth month and in the course of his remarks states that the "open uriniferous tubules" (straight collecting tubules) divide in the cortex, after having given off several branches in the medulla, into four to six side branches, which terminate in the periphery of the kidney either in club-shaped enlargements or with ends which are coiled up several times; these coiled up ends have about the same size as a fully developed Bowman's capsule and glomerulus of a coiled uriniferous tubule. In the coiled up ends of the straight or open tubule, he was not able at first to detect capillary loops and therefore spoke of them as *pseudoglomeruli*. Toldt, in discussing a stage in the development of the uriniferous tubule which corresponds to the S-shaped stage, makes use of this term, although in a foot-note, he calls attention to the inappropriateness of it. Hamburger, after discussing the mode of formation of the S-shaped stage, speaks of it, as a stage designated by Colberg as a pseudoglomerulus, a term which, as Hamburger states, has been adopted by the majority of later writers and is retained by him. The term is misleading and inappropriate and need not be retained, as the mesenchyme and capillary loops found in the concavity of the lower S-curve and recognized as the anlage of the glomerulus, form at this stage only a small part of the entire tubular anlage.

THE GENESIS OF THE DIFFERENT PARTS OF A URINIFEROUS TUBULE FROM THE TUBULAR ANLAGEN OF THE S-STAGE.

The developmental changes undergone by the anlagen of uriniferous tubules, following the stage at which these present the S-shape, affect simultaneously the different parts of the S-shaped structure. In their further growth the tubular portions, embracing the upper S-curve and S-middle piece, elongate, while the bowl-shaped portion, the lower S-curve, enlarges and obtains a deeper concavity; this is accompanied by a proliferation of the vascularized mesenchyme contained within this concavity. We may consider first the changes affecting the tubular portion. This, as may be seen, is relatively fixed at its two ends, on the one hand by its attachment to the collecting tubule, on the other by its attachment to the bowl-shaped structure which contains the vascularized mesenchyme and which early forms a relatively fixed structure. Thus

in its elongation it is forced to acquire secondary curvatures. Of these one is fairly constant both as to the time of its appearance and as to its location, appearing soon after the S-stage is reached and involving about the middle of the upper S-curve. The convexity of this curve is often, though not always, directed inwards toward the collecting tubule. Soon after the appearance of this curvature or coincident with it, the region of the junction of the S-middle piece and lower S-curve becomes rounded out into an arched tubule with convexity turned upwards, the lower S-curve contributing to its formation. Hence, as previously stated, not all of the lower S-curve goes to form the bowl-shaped structure. A portion of variable length in the region of its junction with the S-middle piece assumes a tubular form and with a portion of the S-middle piece forms the arched tubular segment to which reference is here made. This tubular segment may as a whole be bent forward or backward or may early acquire secondary curvatures. The region of the junction of the upper S-curve with the S-middle piece now forms a distinct loop. This region in the S-stage lies in the sagittal plane of the tubular anlage, as may be seen in *D* and *E* of Fig. 3. Coincident with the developmental changes above referred to, this portion of the tubular anlage becomes turned on its axis to a variable extent, the arm of the loop formed by the lower portion of the upper S-curve being moved backward, so that the plane of the loop now intersects a plane passing as in sagittal section through the portion representing the lower S-curve. The angle formed by these two planes varies in the different anlagen from an acute-angle to nearly a right angle. These changes in form and relative position of the upper S-curve and S-middle piece seen in various degrees of development are shown in the models reproduced in *E*, *F*, and *G* of Fig. 4, *D* of Fig. 5, and *D* and *E* of Fig. 6. *E*, *F*, and *G* of Fig. 3 show the appearance presented by tubular anlagen in these stages when seen in sagittal sections; *E* shows a tubular anlage when the tubular portion deviates but slightly from the sagittal plane, the tubule being cut through its entire length, its lumen appearing, however, only here and there, while in *D* the curvature of the tubule has progressed to such an extent that only a part of it falls within the plane of a sagittal section passing through the middle of the anlage; this is more marked in *G* of this figure. A comparison of these figures with *E*, *F*, and *G* of Fig. 4, reconstructions of the respective tubules, will assist in their interpretation. While the above mentioned developmental changes, which affect the tubular portion of the anlage, are in progress, changes affecting the lower S-curve are also to be observed. These changes consist in an enlargement as a whole of this portion and in a growing upwards of its border, thus deepening its con-

cavity and changing it from a structure having the shape of a segment of a sphere to a hemisphere, the mesenchyme found in the concavity of the shallow structure of the earlier stage proliferating as this obtains a greater concavity, especially noticeable being an increase in the number of the capillary loops. That the epithelial portion of this structure is double-walled is evident from what has been said of its anlage and further development; its border represents the edge along which the outer wall becomes continuous with the inner wall. At about this stage of its development, its inner layer on the side toward the collecting tubule and in the region where it is continuous with the tubular portion of the anlage and at about the level of its border, develops a fold which grows outward, away from the collecting tubule, which fold in its further growth becomes continuous with the border. In sagittal sections, this fold appears as a spur and is seen in *F* and *G* of Fig. 3 and also in certain of the tubular anlagen as shown in Fig. 11. It assists in narrowing the wide opening by means of which the space (occupied by mesenchyme and capillaries) enclosed within the double-walled structure communicates with the outside. This fold further assists in differentiating more clearly this structure of hemispherical shape from the tubular portion of the anlage, in that the former now obtains a continuous border along the entire length of which the inner wall is reflected into the outer wall. This brings the attachment of the tubular portion in connection with its outer wall. The attachment of the tubular portion is always on its inner side, on the side turned toward the collecting tubule.

In a tubular anlage developed to the extent here described, there may be recognized the anlagen of the different parts of a uriniferous tubule, as these are known after its development. Briefly stated, the genesis of the different parts of a uriniferous tubule is as follows: The double-walled hemispherical structure, which develops from the greater part of the lower S-curve, forms the inner and the outer layer of Bowman's capsule (more correctly stated, the outer layer forms the epithelial lining of Bowman's capsule, while the inner layer forms the glomerular epithelium); the space between the two layers, continuous with the lumen of the tubules, forms Bowman's space; the vascular mesenchyme enclosed within this double-walled structure forms the anlage for the glomerulus, the two structures taken together forming a stage in the development of a Malpighian body or corpuscle. The region of the junction of the lower S-curve and the S-middle piece, which, as stated, differentiates into an arched tubular segment, represents the anlage of the proximal convoluted tubule. Between this arched tubular segment and Bowman's capsule there is described a short segment, which is known as

the neck of the uriniferous tubule. At this stage of development this portion of the tube is not clearly defined and is not fully differentiated until the epithelium of the portion which will form the proximal convoluted tubule shows structural differentiation. The region of the junction of the S-middle piece with the lower part of the upper S-curve forms the anlage for the loop of Henle, that portion of the S-middle piece which does not participate in the formation of the proximal convoluted tubule forming the proximal arm of the loop, the lower part of the upper S-curve to the region where appears the secondary curvature, about its middle, as above stated, forming the distal arm of the loop, the junction of the two parts, the loop itself. The secondary curvature of the upper S-curve first to appear forms the anlage of the distal convoluted portion. The upper portion of the upper S-curve from its attachment to the collecting tubule to the region where the distal convoluted portion has its anlage may be recognized as the beginning of the junctional tubule. The epithelium of the entire tubular portion of the uriniferous tubule presents at this stage of development essentially the same structural appearances. The cells throughout are of columnar shape, with a relatively small amount of protoplasm, which, unless the bleaching has been very thorough, retains to a certain extent the nuclear stain, for instance staining lightly in haematoxylin if this stain has been used. The nuclei are relatively large, of round or oval shape, and stain relatively deeply. The lumen throughout is of about the same size and is narrow. The statements here made as to genesis of the different parts of a uriniferous tubule are based on observations made on reconstructions of developing uriniferous tubules, of stages beginning with the S-stage to a time when the metamorphosis has progressed until the different parts of a uriniferous tubule may be recognized. Attention should, however, be again called to the fact that, while models which show these stages of development present a certain similarity of form and arrangement of parts, they differ when studied in detail, as was stated in discussing earlier stages of development.

In the further elongation of the tubular portions of the uriniferous tubules, as these proceed in their development, beginning with a stage as above discussed, the portion which was above mentioned as forming the anlage of the loop of Henle needs first consideration. This portion of the tubule is found in the S-stage and some little time after, immediately above the concavity of the lower S-curve, the anlage of Bowman's capsule, as may be seen in reproductions of reconstructions showing this stage. As this loop elongates, it either grows a little to one side (forward) and then elongates downwards toward the pelvis of the

developing kidney, as shown in *F* of Fig. 6, or grows downward from the beginning and in doing so appears to push aside the anlage of Bowman's capsule, which in being pushed aside is turned on its axis so that its posterior border attains a higher level than its anterior border. This is very well shown in *G* of Fig. 6, also, though not quite so clearly, in *E* of Fig. 5. While the loop of Henle is thus developing, the portion which is to form its ascending or distal limb remains in close proximity and on its inner side to the developing Bowman's capsule, after crossing the place where this is joined to the tubular portion which forms the anlage for the proximal convoluted tubule, the descending or proximal limb of the loop lying to the inner side or slightly in front of the distal limb. The two arms of the loop are generally, from the time when they may be clearly recognized, parallel or nearly parallel; now and then the descending limb may be twisted slightly over the ascending limb, as in *F* of Fig. 5. In the models here reproduced, which show the early stages in the development of the loop of Henle, it will be observed that the loop of Henle grows down in front of the anlage of the Malpighian corpuscle or in front of the tubular segment attached to it. This may be accepted as a general law and holds good also for the tubules developing to the left of the collecting tubules, the ones shown in the figure all developing to the right of the collecting tubules. This statement I shall desire to amplify somewhat in discussing more fully the relations of the developing uriniferous tubule to the collecting tubules when I shall also consider Stoerk's observations on this point. Coincident with the formation of the definite anlage of the loop of Henle, as here given, the portions of the tubule which I have designated as destined to form the proximal and distal convoluted portions also increase in length, especially the former, which grows upward and acquires two or three secondary curves. It begins at the developing Malpighian corpuscle and extends upward behind the anlage of the loop of Henle, then arches forward between the collecting tubule and the other parts of the respective uriniferous tubule, then passes downward to become continuous with the descending limb of Henle's loop. Just before the loop is reached, it often shows a distinct flexure, which is fairly constant and characteristic, appearing also in older stages. The curvature which forms the anlage of the distal convoluted portion does not grow in length as rapidly as that part which forms the proximal convoluted portion. The curvature becomes more pronounced as development proceeds and may obtain one or more secondary bends, or may show in much older stages than here discussed a single bend. It may here be added that there is observed a considerable variation as to the relative length of this

portion of a developing uriniferous tubule. Of two uriniferous tubules of apparently the same stage of development, the distal convoluted portion of one may be as long again as that of the other. This is to a certain extent true of uriniferous tubules reconstructed from embryos of the same species, more clearly seen when those reconstructed from different species are compared. As a rule in reconstructions of uriniferous tubules showing early stages of development obtained from human embryos, the distal convoluted portion is relatively longer than in such obtained from cat and rabbit embryos. Compare for example *J* of Fig. 4 with *H* of Fig. 6, two tubules showing about the same stage of development. The distal convoluted portion of a uriniferous tubule is generally found just above the Malpighian corpuscle of the respective tubule, a relation which is fairly constant even for later stages of development, and will receive further attention in considering these. During the time in which tubular portions of uriniferous tubules develop as above described, the anlagen of the Malpighian corpuscles of hemispherical shape change to corpuscles of spherical shape. This is accomplished by a growing upwards and a turning in of the border of the double-walled epithelial structure representing the anlage of Bowman's capsule and by a growing outwards of the fold which arises from its inner wall in the region of its attachment to the tubular portion, this fold being continuous with its border. In this way the opening into the double-walled structure is gradually narrowed until only a relatively small opening remains, at which, as in earlier stages, the inner wall becomes reflected into the outer wall. This opening into the double-walled spherical structure as thus developed and which may now be known as a Bowman's capsule, is situated in its outer and upper portion. During this time, the mesenchyme and vessels recognized as the anlage of the glomerulus differentiate into a definite glomerulus, the small opening leading into the cavity of Bowman's capsule serving for entrance and exit of the afferent and efferent vessels of the glomerulus, these being accompanied by a small amount of connective tissue. A Malpighian corpuscle of this stage of development presents essentially the same shape as a fully developed corpuscle and differs from those found in post-foetal life, aside from certain details in cell differentiation, only in being smaller. The relative degree of development of a Malpighian corpuscle and the tubular portion of a uriniferous tubule, when the extent of the development of the former is compared with the extent of the development of the latter, varies somewhat for different tubules reconstructed from embryos from the same species of animals; more so when developing uriniferous tubules obtained from embryos of different

species of animals are compared. In human and pig embryos the Malpighian corpuscles develop relatively early, when considering the extent of the development of a given uriniferous tubule and may assume a nearly spherical form before the portion of the tubule which forms the anlage of the loop of Henle is clearly differentiated. This, H and I of Fig. 4 may serve to illustrate. On the other hand in cat and rabbit, developing uriniferous tubules are often met with in which the anlage of the loop of Henle is clearly made out, before the Malpighian corpuscles have progressed in development beyond the stage in which they are of hemispherical shape, with the epithelial portion in the form of a double-walled cup, as for instance shown in *E* of Fig. 5, and *F* and *G* of Fig. 6.

Attention may yet be called to the fact that Malpighian corpuscles develop in about the location in which they have their anlage. Throughout the period in which new tubules are formed, new generations of tubules and Malpighian corpuscles develop outside (toward the periphery of the kidney) of those previously formed, which makes it appear as though the Malpighian corpuscles of the older generations of uriniferous tubules sank down into the deeper parts of the parenchyma of the kidney as this developed. Herring, who has called especial attention to this fact, expresses himself as follows: "The Malpighian corpuscles seem to be fixed structures at an early period and do not move their position as do the tubules during their further growth. The early fixation seems to be due to the density of the connective tissue around the Malpighian body and it is likely that a certain part always remains constituting the framework." The size of these structures when compared with the other portions of the tubular anlagen, the compactness of the glomerular anlage, when compared with the surrounding mesenchyme, but especially the early development of a definite vascular supply to the glomerulus, all appear to me to form factors which assist in the fixation of the developing Malpighian corpuscle.

At about the time when the developing Malpighian corpuscles have reached a stage of development in which they resemble in shape and to a certain extent in structure, fully developed Malpighian corpuscles, there is observed a cellular differentiation in that portion of the tubules which we have designated as the anlage of the proximal convoluted portion. Beginning with the region of attachment of this portion of the tubule to the Malpighian corpuscle and proceeding for the remainder of its extent it may be observed that the protoplasm of the epithelial cells increases in quantity and becomes clearer and now takes more readily a protoplasmic stain, so that in sections stained with haematoxylin and eosin or erythrosin, the protoplasm of the cells appears tinged with red.

At the same time, the nuclei take a position in the basal portion of the cells, so that the lumen becomes surrounded by a distinct margin of clear protoplasm. The nuclei also stain less deeply than in earlier stages of development and present a vesicular appearance. The lumen of this portion of the tubule, which, before the cell differentiation is observed, is relatively narrow, becomes prominent. This cell differentiation extends the entire length of the proximal portion and for a variable distance along the descending arm of Henle's loop, as developed at this stage. This cell differentiation is shown in *I* and *J* of Fig. 3. In the former is shown about the distal one-half of the proximal convoluted portion of the tubule represented in reconstruction in *I* of Fig. 4, and its junction with the remainder of the tubule, the epithelium of which shows as yet no differentiation. In the latter is shown a loop of Henle cut through its entire length about the upper half of the descending limb of which shows an epithelium with clear protoplasm and nuclei with basal position. The proximal convoluted portion of the tubule is further seen in cross section just to the right of the upper end of the descending limb of Henle's loop as seen in the figure; this also shows the characteristic cell differentiation. In the immediate vicinity of the Malpighian corpuscle, where the tubule becomes continuous with the outer layer of Bowman's capsule, this cell differentiation is not so pronounced as in other parts of the proximal convoluted portion, nor do the cells of this region attain the same size as in other parts of the tubular segment; the lumen likewise is not so well developed. Consequently the tubule presents here for a short though a variable distance a smaller diameter than in other parts of the proximal convoluted portion and may now be recognized both by reason of its smaller size and by its structure as what is known as the neck of the uriniferous tubule. At this stage of development, the remaining portions of the uriniferous tubule, the greater part of the loop of Henle, the distal convoluted portion, and the junctional tubules present essentially the same structural appearance seen in the earlier stages of development.

The so-called junctional tubules of the uriniferous tubules elongate as these develop with the growth of the kidney. As has just been stated, a Malpighian corpuscle develops in about the location of its anlage; the proximal and distal convoluted portions of a tubule of which such a corpuscle forms a part, differentiate in its immediate vicinity. As the developing kidney grows in size, the collecting tubules elongate, their ends being always found just under the capsule, and as the junctional tubules are inserted at the peripheral ends of the collecting tubules (the exceptions I shall note) the collecting tubules, as they elongate, carry with them the ends of the junctional tubules; those for the uriniferous

tubules first developed must then traverse the greater part of the cortex, so far as developed, in order to reach the peripheral ends of the collecting tubules. (See Stoerk, page 304.)

A detailed description of the several models, on which is based the account here given of the early developmental stages of uriniferous tubules, is

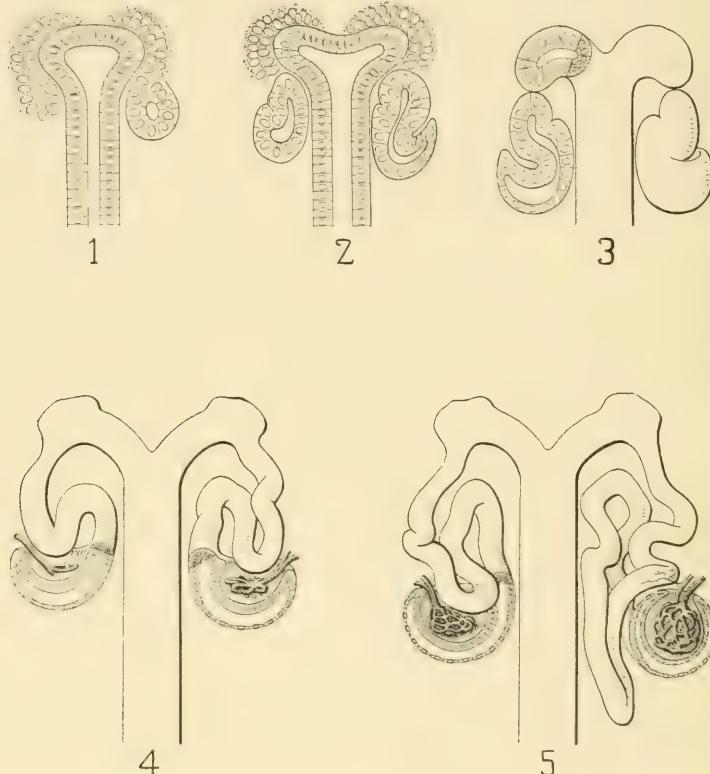


FIG. 8. Semidiagrammatic figures of the anlage and differentiation of renal vesicles and early developmental stages of uriniferous tubules of mammals. 1 and 2, anlage and successive stages in the differentiation of renal vesicles, as seen in sagittal sections; 3, section and outer form of tubular anlage before union with collecting tubule at the beginning of S-shaped stage; 4 and 5, successive stages in the development of the tubules, Bowman's capsule and glomerulus beginning with a tubular anlage showing a well developed S-shape.

obviated, it seems to me, by the number of illustrations of these stages here presented. My own observations and the conclusions reached concerning the anlage and early developmental stages of the uriniferous tubules and the genesis of the different parts of these tubules I have summarized by way of a series of diagrammatic figures grouped under Fig. 8.

Brief mention may yet be made of the conclusions reached by former observers who have considered the genesis of the different parts of the uriniferous tubules. Toldt, who was the first to recognize clearly the S-shaped stage in the development of the uriniferous tubule, a stage in which the anlage is spoken of as a pseudo-glomerulus, does not give a clear account of the anlage of the tubular portion. I find in his account the following statement, which I shall give in his own words: "Bemerkenswerth ist ferner, dass die Windungen des Canälchens constant den der Nierenoberfläche zugewendeten äusseren Theil des Pseudoglomerulus einnehmen, während das schalenförmige Ende des Canälchens stets in dem Abschnitte desselben gelegen ist, welcher nach dem Inneren der Niere zu sieht." (Page 134.) This statement taken in connection with Toldt's account of the development of the Malpighian corpuscle, I interpret as meaning that he regards Bowman's capsule (and glomerulus) as developed from the lower curve of the S-shaped structure while the remaining parts of the uriniferous tubules are developed from the tubular portion of the "pseudoglomerulus" and not, as is now and then stated, that Toldt regards the S-shaped tubules (pseudo-glomeruli) as merely the anlagen of the Malpighian corpuscles. Haycraft, in his account of the anlage of the different parts of the uriniferous tubule, makes reference to a figure (Fig. 8 of his article), which he describes as "a high power view of the first formation of a urinary tubule from a primary renal vesicle," which figure I have interpreted as showing only a portion of a tubular anlage. He states that "each little sprout from a renal vesicle will, in other series of older embryos, be seen to elongate, the bulging portion marked *H* will grow down toward the primary pelvis to form the convoluted tubules and the loop of Henle, the part at *m* marks the formation of the future Malpighian body." The bulging portion marked *H* as shown in his figure refers to the region of the junction of the S-shaped tubular anlage with the ampulla of the collecting tubule, *m* marks the end of the tubule as seen in the figure. Golgi's account of the development of the uriniferous tubules, elucidated by his well known semidiagrammatic figures of developing renal tubules of mammals is here given as presented by Minot (Page 510), leaving out the reference letters. "The different parts of the S-shaped tubule have each their fixed destiny. The end of the S (in the diagrams the lower part) receives the vascular loop, which gives rise to the blood-vessels of the future glomerulus; the lower limb of the S elongates enormously and forms the first division of the convoluted tubule including the loop of Henle; the upper limb of the S also elongates very much—though less than the lower limb—and is the anlage of the second division of the convoluted tubule; where the two join, the tubule passes close to the Malpighian corpuscle and seems to be intimately attached to it." Except perhaps for the fact that I have differentiated more clearly the different parts of a tubular anlage of an S-shape and am thus able to give more explicitly the genesis of the different parts of a uriniferous tubule, my own account may be considered as confirming Golgi's account as here given. Hamburger states that the first portion of the coiled uriniferous tubule to differentiate (leaving out of consideration Bowman's capsule) is the loop of Henle, which has its anlage in that portion of the S-shaped tubule which is taken up by the bowl-shaped anlage of Bowman's capsule ("welche eigentlich durch die von der Schale aufgenommene Biegung des S-förmigen Canälchens vorgebildet ist"). The portion which extends into the concavity of the lower S-curve in the region of the junction of the upper S-curve and S-middle piece, which I have also regarded as the anlage of the loop of Henle (I am not certain that I have interpreted the above statement of Hamburger correctly, as the account is not quite clear to me). He also states that the distal convoluted portion (Schaltstück) is differentiated early, before the proximal convoluted portion (Tubulus contortus) has acquired any coils. The latter portion, he states, is developed from the third, the most distal limb of the S-shaped tubule. It should be recalled that Hamburger recognizes in the S-shaped stage (pseudoglomerulus) a bowl-shaped structure and an S-shaped tubular portion (see page 36 of this article) much as described by Stoerk for his secondary S-stage. Hamburger, in speaking therefore of the third or distal limb of the S-shaped tubule refers to the curved tubular segment which is in continuity with the anlage of Bowman's capsule; this would represent the tubular segment which develops from the region of the junction of the lower S-curve and S-middle piece, which I have also regarded as the anlage of the proximal convoluted portion. Schreiner recognizes in the S-shaped tubular anlage essentially the same regional differentiation leading to the formation of the different parts of the uriniferous tubule as given by me, as may be seen by a study of his Fig. 114, to which especial reference is made in his account. Stoerk's observations on the mode of formation of the different parts of the uriniferous tubules need to be considered somewhat more fully, as he is the only one of previous workers who has made extensive use of reconstruction methods in the study of the subject. It will be remembered that he recognizes a primary and a secondary S-stage in the development of the uriniferous tubules. As the Malpighian corpuscle completes its development, the S-shaped tubule of the secondary S stage elongates and acquires from four to five "short, plump windings." The epithelium of the tubular portion, which up to this stage, has shown the same structure throughout, now shows a differentiation, beginning with the attachment of the tubule to the outer layer of Bowman's capsule and extending for one to two windings of the tubule. The cells here obtain more protoplasm, which becomes clearer and the nuclei assume a basal position. This cell-differentiation, as described by Stoerk, both as to the time of its appearance and as to the region of the tubule affected is about as given by me. About this time, there develops from the coiled tubular portion a loop which grows downward over the Malpighian corpuscles to form the loop of Henle. Concerning the location of this loop, Stoerk has this to say: I use his own words: "Es lässt sich von vornherein durchaus nicht sagen, welche von den ursprünglich untereinander ganz gleich aussehenden Windungen zu diesem Anwachsen zur Schlinge bestimmt ist, solange in allen das Epithel ein dunkles ist; erfahrungsgemäß ist es meist die zweite nach dem Abgang vom Malpighischen Körperchen." That the portion of the tubule

which is to form the anlage of the loop of Henle can be more definitely located than this statement would lead one to think I believe my models will show, especially those obtained from the cat and rabbit. In models of developing uriniferous tubules obtained from human embryos, in which the Malpighian corpuscles develop relatively early and the loop of Henle at a correspondingly later period, the portion of the tubule which is to form the loop of Henle is often not easily located; yet even from a study of these models, I have gained the conviction that the loop has its anlage in what has been termed the region of junction of the upper S-curve and S-middle piece. It is of interest to note that Stoerk states that experience shows that it is generally the second loop after the tubule leaves the Malpighian corpuscles, which forms the anlage of the loop of Henle; this corresponds, in the majority of the models, with the region of the junction of the upper S-curve and S-middle piece and confirms, therefore, the view I have expressed.

The majority of the observers who have considered the development of the Malpighian corpuscle have in whole or in part followed Toldt, who considered Bowman's capsule formed by an invagination, from one side, of the blindly ending spherical dilation of the developing uriniferous tubule, mesenchyme and capillaries participating in the invagination and forming the anlage for the glomerulus. Toldt compares this process of invagination to the pressing in of one side of a hollow rubber ball until the side pressed in comes in contact with the other side. The glomerulus develops in the concavity thus formed. Kölliker, Pye, Janosik, Nagel, Minot, Schultze, Gerhardt, and Strahl may be mentioned as accepting this view, although it is not always clear from their accounts to what extent they consider the formation of the double walled cup, the anlage of Bowman's capsule, due to an actual invagination rather than to a process of growth on the part of the anlage of Bowman's capsule by means of which the anlage of the glomerulus becomes surrounded. Gerhardt, for instance, states that it is difficult to say whether the blind end of the uriniferous tubule plays an active or a passive rôle in the formation of Bowman's capsule, most likely both, in that, while the capillary loops grow inward, the tubule takes an active part in the formation of Bowman's capsule. A more correct interpretation of the process of development of Bowman's capsule and the glomerulus is given by Herring, although he missed the anlage of the part which is destined to form Bowman's capsule, as has been previously stated. He states that "the first appearance of the cavity of Bowman's capsule is a narrow slit and never a vesicle in the human kidney. Toldt's description of the driving in of one wall by capillaries is not an accurate one. At this stage there are no capillaries in the glomerulus." "The further growth in size of the glomerulus may be described as an invagination, but it is not an invagination of the kind usually supposed. None of it takes place at the expense of the other side as occurs in Toldt's illustration of the rubber ball. It is rather an increase in size by proliferation of its own constituents; the base remains in the same position and is wide at first but gradually narrows, the narrowing being brought about by the formative action of large cells covering it." The formation of the lower S-curve, the anlage of Bowman's capsule, has been correctly given by Schreiner, as has been stated, and in this my own observations confirm him. By way of summary it may be stated that the two layers of the lip-shaped fold, which is separated from the outer, lower part of the renal vesicle, and which forms the anlage of the lower S-curve, are from the beginning nearly in contact, the fold presenting a slight concavity above and a corresponding convexity below. As the anlage of the lower S-curve proceeds in its development, it increases in size and assumes the shape of the bowl of a spoon, with a distinct border except where attached to the tubular portion of the anlage; the handle of the spoon, which must be thought of as bent so as to form the greater part of an S. At about this time, a small amount of mesenchyme may be found in the concavity of the lower S-curve, at first without capillaries, these growing in from without. In its further development, this double walled structure, which represents the segment of a sphere, changes to a hemisphere by a growing upwards of the border, the mesenchyme proliferating and the capillaries increasing in number so as to fill the concavity as this develops. By a further growing upwards and a turning in of the border and by the formation of a fold on the inner layer, the hemispherical structure changes to a spherical structure, Bowman's capsule and the glomerulus being thus completed. That Bowman's capsule with its outer and inner layer is developed by growing upwards and ultimately by a turning in of the border of the structure with shallow concavity as seen in the lower S-curve when this is first recognized, both sections and reconstructions seem to me to show. In sections of a developing Bowman's capsule, both the inner and outer layer for a short distance from the border and along its entire length show undifferentiated embryonic cells indicating the growing zone. This may be seen in the sketches shown in Fig. 3. This view of the development of the Malpighian corpuscle is the one taken by Stoerk, who has given correctly the main features of its development, leaving out of consideration the anlage of the lower S-curve, the formation of which he hardly considers, and the formation of the fold which assists in transforming the hemispherical into a spherical structure; this fold he recognized but believes it to be developed from the middle S-piece of the primary S, which, as has been stated, he regards as being taken up into the anlage of Bowman's capsule, while the primary S-stage is changing into the secondary S; in my own account, I have endeavored to show that it develops as a fold of the inner layer of the anlage of Bowman's capsule independent of the S-middle piece.

Throughout the period in which new tubules are formed, new generations of uriniferous tubules develop outside (toward the periphery of the kidney) of those previously formed, the latter thus coming to lie relatively

deeper down in the parenchyma of the kidney, as this increases in size; the tubules showing the greatest development are therefore formed nearest the pelvis of the developing kidney. To show the relative size and the shapes of uriniferous tubules and tubular anlagen developing from different generations of renal vesicles as also the relative size, shape and extent of branching of collecting tubules and their relation to the former, for a developing kidney of which the most fully developed tubules present about the size and shape of those shown in *J* of Fig. 4, and *H* of Fig. 6,

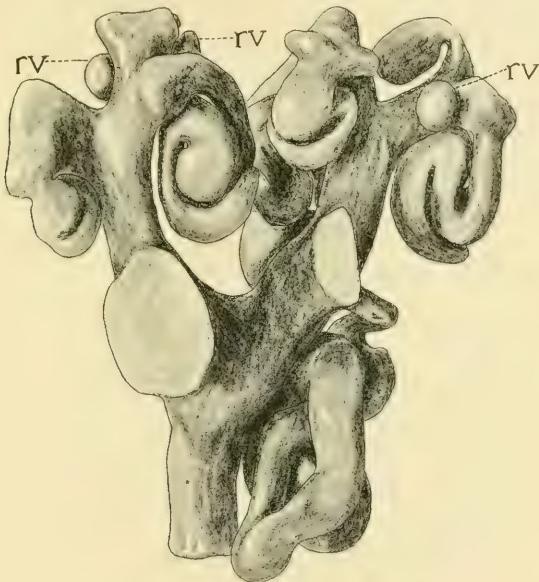


FIG. 9. A model of a large or primary collecting tube, cut just above its origin from the pelvis of the kidney with the collecting tubes resulting from four successive dichotomous divisions, with three renal vesicles, five tubular anlagen in the S-shaped stage and two uriniferous tubules in early stages of development; from the kidney of a human embryo (No. 7), measuring 5 cm. in length. $\times 133.$ *rv*, renal vesicles.

these representing the oldest stages thus far discussed, I have inserted Figs. 9 and 10. In each of these figures is reproduced a model of a primary collecting duct, beginning with a region a little above its place of origin from the pelvis of the kidney and showing the successive divisions of the same to the ampullar enlargements of the end branches, together with the renal vesicles, tubular anlagen and uriniferous tubules which had developed therewith. Fig. 9 represents these structures as observed in a human embryo 3 cm. in length (embryo No. 7). In this figure, for the sake of clearness, only a part of the model is reproduced. The cut

surfaces recognized by their shading represent the origin of two branches, similar to those reproduced. The collecting tubules are at this stage of development relatively large with wide lumen. The one here shown

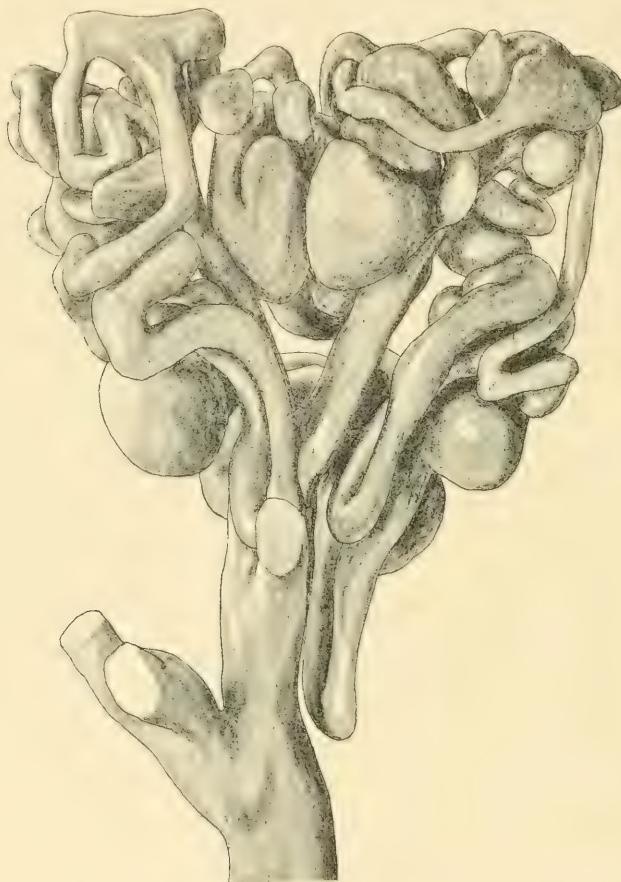


FIG. 10. A model of a primary collecting tubule, cut just above its origin from the pelvis of the kidney, showing six successive dichotomous divisions. Only one of the four main branches of the second generation of branches is here shown in full, together with renal vesicles, tubular anlagen and uriniferous tubules developing in connection with this branch. From the kidney of a human embryo (No. 11), measuring 6.5 cm. $\times 133$.

presents four successive dichotomous divisions, the end-branches showing ampullar enlargements. The pelvis of this kidney and the collecting tubules here shown are lined by a single layer of columnar cells. The figure shows further three renal vesicles, *rv*, five tubular anlagen in the S-stage, seen from different aspects and showing different degrees of

development and two uriniferous tubules of a stage of development in which the different parts of a uriniferous tubule may be readily made

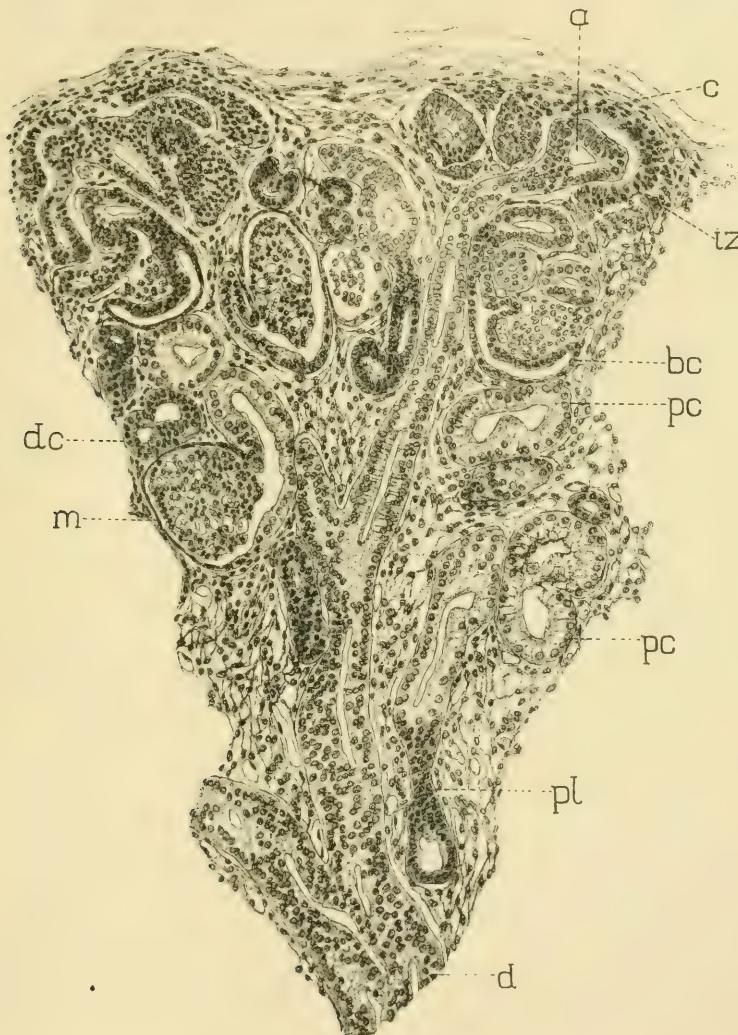


FIG. 11. A portion of a longitudinal section of a kidney of a human embryo (No. 11) measuring 6.5 cm. (This section is the most typical of the series of sections used in making the preliminary drawings from which was made the model shown in Fig. 10.) $\times 133$. *C*, capsule; *d*, collecting tubule; *pl*, proximal or descending limb of Henle's loop; *m*, Malpighian corpuscle with neck and a portion of proximal convoluted tubule; *bc*, anlage of Bowman's capsule and a glomerulus showing fold of inner wall of capsule; *dc*, distal convoluted tubule; *a*, end branch of collecting tubule and ampulla; *iz*, inner zone of metanephrogenic tissue; *pc*, proximal convoluted tubule.

out and the respective Malpighian corpuscles are nearly developed; one of these is on the rear side of the model as sketched and shows only in part, between the two prominent diverging branches. The relation of these structures to each other and to the collecting tubule is shown with sufficient clearness in the figure to obviate fuller discussion. In Fig. 10, is shown a similar reconstruction for a human embryo of 6.5 cm. length. The long diameter of the kidney from which this reconstruction was made measures 5 mm. after fixation. The collecting tube reconstructed presents five and in some branches six successive dichotomous divisions. Of the four branches resulting from the second division, only one was reconstructed in full, the others are represented as cut soon after they are separated. That the collecting tubules grow in length along their entire extent as the kidney develops is shown by the fact that a greater distance intervenes between the successive branches than is shown in the former stage; the angle at which the branches meet is for the older stage more acute than for the earlier stage. During this growth in length of the collecting tubules, they obtain a smaller diameter and consequently a smaller lumen; especially is this true of the later generations of branches. In connection with the one large branch here fully reconstructed, there are found in the model five renal vesicles and 14 tubular anlagen and uriniferous tubules in various stages of development, only a portion of which could be represented in a sketch presenting one view of the model. The complexity of this model is such that a reproduction of it can give only in a general way the shape, size, and relations of the various structures modeled and this can be shown in one figure quite as clearly as in several figures drawn from different aspects of the model. In Fig. 11 is reproduced one of the most typical sections of the series from which the model shown in the former figure was made; it falls in a plane which passes through about the middle of the model. It will serve to show the structure and cellular differentiation of certain representative tubules and tubular anlagen shown in the reconstruction; a comparison of the two figures will enable the identification of the respective parts, as represented in each figure, as they are drawn to the same scale. In the section reproduced, a collecting tubule was cut through its entire length and may be seen to end in characteristic ampullar enlargement. The epithelium lining the collecting tubules is here shown as a single layer of columnar cells which in the primary collecting duct presents the appearance of a pseudostratified epithelium. In each of the two models (Fig. 9 and 10) all of the renal vesicles, tubular anlagen and uriniferous tubules, with the exception of one uriniferous tubule, are associated with or attached to the ampullar enlargement of the end branches of the

collecting tubules. The one tubule of each model having a different attachment is attached to the collecting tubule; in the model showing the younger stage of development, in the region of the second division, in the other model, in the region of the third division. I have observed this condition only in the human embryos. In the kidney of the cat and rabbit of about the same stage of development, as shown in these models, all the uriniferous tubules and tubular anlagen are attached to the ampullar enlargements of the end branches of the collecting tubules. Hamburger, who has made observations on the mode of attachment of the "coiled uriniferous tubule" to the "straight collecting tubule" on certain animals, has reached the following conclusions: He speaks of the branches of the straight collecting tubules which result from the last division of these (as seen in the fully developed kidney) as the "terminal collecting tubules" and finds that in the beef and the mouse and generally also in the rat these terminal collecting tubules take up the ends of the coiled uriniferous tubules and only seldom (in the rat) do these terminate in the straight collecting tubules proximal to the terminal branches, while in the pig a greater number of the coiled uriniferous tubules end in the straight collecting tubules and that these further form arcades, with convexity upwards, which also serve for the insertion of the coiled tubules. The fact that at a relatively early stage in the development of the human kidney certain uriniferous tubules end in the collecting tubules proximal to their terminal branches indicates that this mode of termination obtains in post-uterine life, while the fact that in cat and rabbit embryos of about 2.5 cm. length, all the tubules end in the terminal branches would go to show that even in the fully developed kidneys of these animals, the great majority of the uriniferous tubules end in this way. Positive statements concerning this point I am at present unable to make, as I have not reconstructed collecting tubules throughout their whole extent for stages older than given in Fig. 10.

Figure 9 will also serve to show that the collecting tubules in their growth and successive divisions resulting in the formation of new branches, do not in these successive divisions divide in the same plane, but with some degree of regularity in alternate planes. The configuration resulting from the last two divisions of a collecting tubule during the time when these form new branches, would resemble two Y's the stems of which are joined to form a single stem, the four arms of which project into four quadrants of a circle. The diagram given in Fig. 12 may serve to make this clear. Uriniferous tubules develop in connection with each of the four end branches thus formed. In each of the two tubules developing in connection with the two anterior end branches, as

given in the figure, the loop of Henle grows down in front of the Malpighian corpuscle and the first portion of the proximal convoluted tubule belonging to each tubule. Thus these conform to the general law as previously stated. In each of the two tubules which develop in connection with the posteriorly placed end branches, while these hold the position given in the figure, the loop of Henle grows down behind the Malpighian corpuscle and the first portion of the proximal convoluted tubule. If now the whole configuration as shown in the figure be turned through an arc of 180° , thus bringing the two posteriorly placed tubules in front, these will show the same arrangement and relations of parts

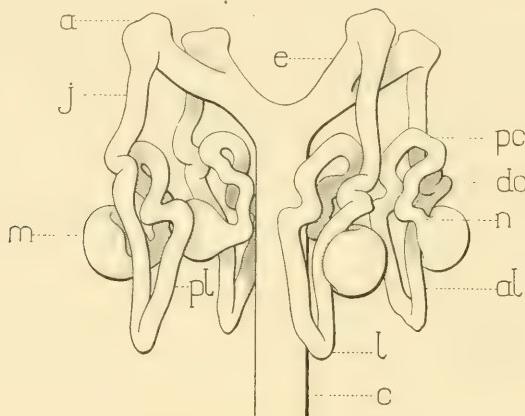


FIG. 12. Semidiagrammatic figure given to show the relations the uriniferous tubules bear to the collecting tubules and end branches as observed a short time before the middle of embryonic life. Not all the uriniferous tubules would show in such a configuration about the same degree of development as here represented. *c*, collecting tubule; *e*, end branch of collecting tubule; *a*, ampulla; *m*, Malpighian corpuscle; *n*, neck; *pc*, proximal convoluted tubule; *pl*, proximal or descending limb of Henle's loop; *al*, ascending or distal limb of Henle's loop; *dc*, distal convoluted tubule; *j*, junctional tubule.

as was shown by the two tubules which previously occupied the anterior position; that is to say, the loop of Henle now passes down in front of the Malpighian corpuscle, while in the other two tubules, those which previously held the anterior position and now occupy a posterior position, the loop of Henle now passes down behind the Malpighian corpuscle. In stating, therefore, the general law that the loop of Henle in its development and elongation passes down in front of the Malpighian corpuscle and the first part of the proximal convoluted tubule, cognizance must be taken of the relationship of a given uriniferous tubule to the collecting tubule in which it ends. The first part of a proximal convoluted tubule, beginning with its attachment to the Malpighian corpus-

cle and embracing a tubular segment of variable length, passes between the loop of Henle and the collecting tubule to which said tubule is attached. If one, therefore allows the collecting tubule to which a uriniferous tubule is attached, irrespective of its other relations, to mark the posterior aspect of said tubular configuration, it may be said that the loop of Henle passes down in front of the first part of the proximal convoluted portion of the tubule, near its origin from the Malpighian corpuscle and this would also assume a position in front of the corpuscle. I can not, therefore, agree with Stoerk when he states, in connection with the statement which I have previously quoted (see page 51) in which he refers to the fact that it was not possible to state which of the four or five loops of the secondary S formed the anlage for the loop of Henle, that—and here I use his own words—"Der Mangel an Gesetzmässigkeit ihrer Bildung äussert sich auch darin dass sie an zwei zum gleichen Sammelrohr gehörigen Bildungen an der einen vor, an der anderen hinter dem Malpighischen Körperchen herabfallen kann (Model L und F auf der Tafelabbildung)." Reference is here made to the downward growth in its development of the loop of Henle. In model *L* of this plate, the loop of Henle is well developed and conforms in its relations with those give in my own descriptions of this portion of the uriniferous tubule. The tubule shown in model *F* of this plate is so little developed that an interpretation of it is not justifiable without seeing the model itself; I will, therefore, not attempt a discussion of it. In all the uriniferous tubules reconstructed by me, the loop of Henle is seen to pass down in front of the Malpighian corpuscle or the proximal convoluted tubule in the vicinity of its attachment to the corpuscle—and this applies to the older as well as to the younger developmental stages modeled—if by in front is meant that side of the tubular complex turned away from the collecting tubule to which a uriniferous tubule is attached as explained above and as may be seen from the diagrammatic figures given.

DIFFERENTIATION OF THE EPITHELIUM OF THE LOOP OF HENLE.

We may now return for brief consideration to the stage of development represented by the tubule shown in *J* of Figs. 3 and 4, representing the oldest stage thus far discussed. This tubule represents one in which the Malpighian corpuscle shows a spherical form and may be regarded as fully developed; the loop of Henle is clearly recognized and presents the relations described for this portion of the uriniferous tubule, the proximal convoluted portion and about one-half of the proximal or descending limb of Henle's loop show an epithelium with clear protoplasm and nuclei with basal position; the cells lining the remaining portions of the

tubule show as yet no specific differentiation, but present the appearances shown by the epithelium lining the entire tubule at an earlier stage of development. In their further development, such tubules increase greatly in length. This growth in length affects all parts of the tubule, though not to the same extent, the proximal convoluted portion elongating more than the distal convoluted portion, the loops of the former becoming more pronounced and new ones forming. The chief increase in length is, however, observed in the two arms of Henle's loop; this, in elongating, grows toward the pelvis of the kidney. While thus elongating, a characteristic cellular differentiation is observed in the proximal or descending limb of Henle's loop, beginning with that region of this portion of the tubule which is marked by the termination of the cells with clear protoplasm and extending to near the loop itself—the region where the descending limb becomes continuous with the ascending limb—though never involving the loop itself. The epithelium lining this portion of the descending limb of the loop, differentiates into a flattened epithelium with flattened elliptical nuclei. The protoplasm of these cells shows very little granulation. The tubule obtains in this portion a smaller diameter than in other parts; this is more clearly seen at a later stage of development, some little time after the epithelial differentiation here mentioned may be clearly recognized. The size of the lumen of this portion of the tubule remains about the same as that of other parts. Coincident with this change of cell structure, as observed in the descending limb of Henle's loop, the cells lining the loop itself, the ascending limb, the distal convoluted portion and for a variable distance in the junctional tubule, undergo a change in structure. The protoplasm of the cells lining these portions of the tubule increases in quantity and acquires a granular, often faintly striated appearance and shows an affinity for protoplasmic stains (eosin and erythrosin), these portions then staining more deeply than the proximal convoluted tubule and the proximal arm of Henle's loop. The cells may be described as being of cubical or low columnar shape, with nuclei of spherical or slightly oval form and placed centrally in the cells. The diameter of these portions of the uriniferous tubules is greater than that of the proximal arm of Henle's loop, showing the flattened epithelium, though less than in the proximal convoluted portion. The epithelium lining the different parts of a uriniferous tubule will receive further consideration in discussing more fully developed uriniferous tubules. In Fig. 13, A, is shown a model of a uriniferous tubule in which the epithelial differentiation of the different parts of the tubule, as here described, may be clearly recognized. The tubule shown represents one which measures 2.5 cm., of which just about one-half falls to the loop

of Henle. (This measurement and others giving the length of uriniferous tubules are obtained from models, the length of the tubule as represented in the model being divided by the magnification at which it was made — 400). In *B* of this figure is shown one of the sections of the series of sections of this tubule from which the model was made; it presents with other parts, a section of the distal part of the proximal convoluted portion and its continuity with the proximal arm of Henle's loop, showing the flattened epithelium, which is cut through its entire length to the immediate vicinity of the loop itself. The transition from the clear epithelium with basal nuclei of the proximal convoluted portion and the first part of the descending limb of Henle's loop to the flattened epithelium of the remainder of the descending limb of Henle's loop, is here clearly shown. The ascending limb of Henle's loop shown in the figure does not belong to the tubule shown in the reconstruction, but to an adjacent tubule also in part reconstructed and of about the same shape and stage of development as the one shown in *A* of this figure, here added to enable a comparison between the epithelial lining of the two arms of Henle's loop as observed at this stage of development. It may be emphasized for purposes of further discussion that the parts here designated as representing the proximal and distal arm of Henle's loop were thus designated after reconstruction of the two tubules of which they form a part.

The account here given of the differentiation of the epithelium of the two arms of Henle's loop differs very materially from that given by Stoerk in his description of the development and cellular differentiation of this portion of the uriniferous tubule. Stoerk states that at a time when the loop of Henle is as yet very short, the clear epithelium which characterizes that portion of the uriniferous tubule destined to form the proximal convoluted portion extends to about the middle of the bend, which is to form the loop of Henle, lining therefore its proximal half, while the other half of the loop anlage is lined by the darker epithelium, which lines the remainder of the tubule. (The terms clear and dark epithelium are here used in the sense given them by Stoerk who speaks of "hellem und dunklem Epithel"). As the loop elongates, the part lined by dark epithelium grows more rapidly than the part lined by clear epithelium, so that the dark epithelium takes in the loop and extends for a variable distance into its proximal arm the clear epithelium ending, therefore, a little above the loop. He further states that "The descending arm of Henle's loop is therefore to be regarded as representing genetically and morphologically the end segment of the tubulus contortus of the first order, while the ascending limb represents genetically and morphologically

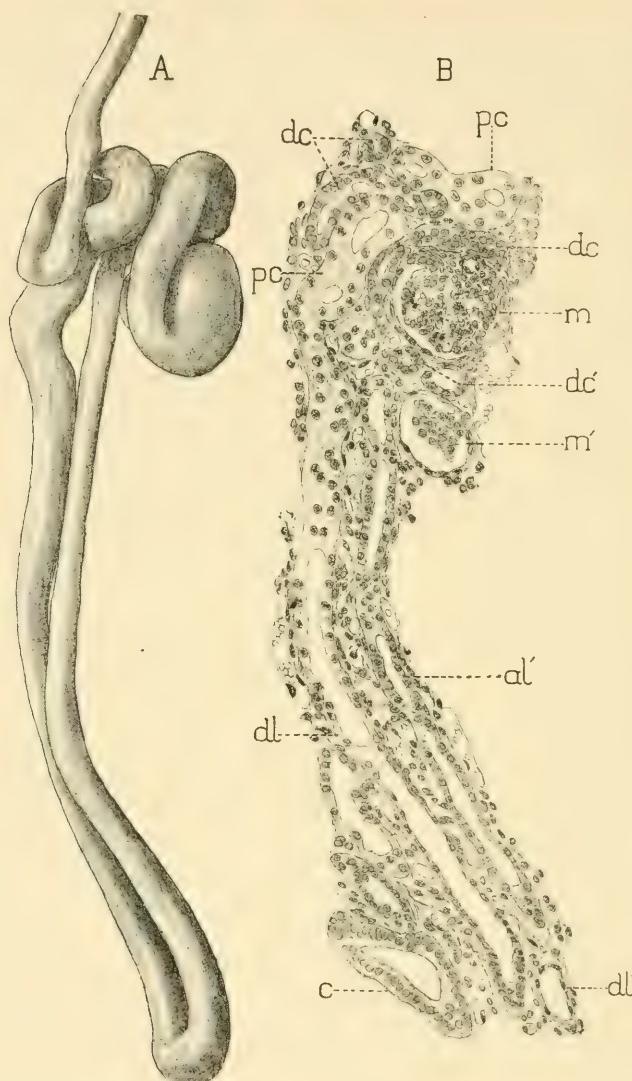


FIG. 13. *A*, uriniferous tubule from the kidney of a human embryo of the seventh month (No. 16). $\times 160$.

B, a portion of one of the sections of the series from which the preliminary drawings used in making the model shown in *A* were made. $\times 160$. *M*, Malpighian corpuscle; *dc*, distal convoluted portion; *pc*, proximal convoluted portion; *dl*, descending or proximal limb of Henle's loop, showing flattened epithelium; *c*, collecting tubule; (all parts of the tubule shown in reconstruction); *al'*, ascending limb of Henle's loop of a second tubule; *m'*, *dc'*, *dl'*, portions corresponding to those designated with like letters without the accent, belonging to a second tubule.

the beginning segment of the tubulus contortus of the second order." The descending or proximal arm of Henle's loop to near its end is therefore, according to Stoerk, lined by an epithelium which is like the epithelium which lines the proximal convoluted portion and presents a diameter of tubule and lumen, which is also like the proximal convoluted portion, while the ascending limb, which is of smaller diameter, is lined by cells having a darker protoplasm and this not only in earlier stages of development, but also in later stages and in post-foetal life, as is apparent from his description and also his diagram given in Fig. 27 of his article. To state clearly Stoerk's position, I shall quote from his summary as follows:

"Das Protoplasma des Kanälchenepithels, von der Insertionsstelle am äusseren Blatt der Bowmanschen Kapsel angefangen bis zur Umbiegungstelle der Henleschen Schleife, wird ziemlich gleichzeitig mit dem Auswachsen der Schleife hell, das lumen dieses Kanälchenabschnittes erweitert. Der helle und weitere Schenkel der Henleschen Schleife ist der absteigende und nicht, wie bisher angenommen wurde, der Aufsteigende." Stoerk also states in discussing his observations on this point that "Dieser völlig einwandfreie und ausnahmslos konstante Befund steht in direktem Gegensatze zu der Darstellung im Schweiger-Seideleschen Schema und, dem seinerzeit Gesagten gemäss, auch im Gegensatze zu dem, was seither zur allgemein gültigen Anschauung über das Verhalten der Henleschen Schleife geworden ist. Dass die falsche Lehre bisher keine Richtigstellung erfahren hat, ist um so verwunderlicher, als eine Reihe von allgemein bekannten Beobachtungen so wohl der normalen wie der pathologischen Histologie auf das Widersinnige in der Sache hätten hinweisen können."

I have quoted thus fully from Stoerk, as his observations (based on reconstructions) on the development and structure of the loop of Henle have led him to conclusions which are so at variance with the generally accepted views concerning the structure of the different parts of this portion of the uriniferous tubule that a clear statement of his position seemed necessary and this could best be given by making free use of his own words. That the older and generally accepted view of the size and structure of the descending or proximal limb of Henle's loop—namely that it represents that part of the uriniferous tubule which shows the smallest diameter, as generally given in the diagrams of uriniferous tubules, beginning with the well-known one of Schweiger-Seidel, and that it is lined by a flattened epithelium—is the correct one and that Stoerk is in error when he states that this portion of the uriniferous tubule presents essentially the same diameter as the proximal convoluted tubule and is lined by a similar epithelium, is shown by those of my models which show advanced stages in the development of the uriniferous tubules. The source of his error can, I believe, be readily shown. If one may be allowed to judge from his figures, he has reconstructed only relatively early stages in the development of the uriniferous tubules. The tubule shown as model *L*, as found in his plates, presents the most advanced stage figured by him. Figure 18 shows in very favorable section a tubule of about the stage of development as that shown in model *L*. In the

tubule figured as seen in section, as is also apparent in references made to it in the text, the clear epithelium found in the proximal convoluted portion extends for about half the length of the descending or proximal arm of Henle's loop. This portion of the tubule has also a greater diameter than is shown by the greater part of the remainder of the tubule figured by him, the latter part being lined by the darker epithelium. The tubule shown in Fig. 18 of his article presents about the same stage of development and cellular differentiation as that shown under *J* of Fig. 3 of this article, and this tubule was selected as showing a stage of development just prior to that in which the epithelium of the proximal and distal arms of Henle's loop shows the specific differentiation described for them. In *A* and *B* of Fig. 13, in which is represented a tubule showing a stage in which the epithelium of all its parts shows characteristic differentiation and represents a stage a little more advanced than the oldest stage figured by Stoerk, it may be clearly seen that that portion of the descending limb of Henle's loop which in *J* of Fig. 3 or in Fig. 18 (Stoerk's) shows the darker epithelium, differentiates as the loop elongates, into the flattened epithelium characteristic of the greater part of the descending limb of Henle's loop. Stoerk, in formulating his conclusions relating to the shape and structure of the descending and ascending limbs of Henle's loop makes use of data gained from a study of uriniferous tubules representing stages of development in which the epithelial differentiation is not as yet complete in all parts of the tubules, notably in the descending and ascending limbs of Henle's loop. It may readily be seen how from such insufficient data, he would be led to the conclusions drawn. If the loop of Henle in either of the tubules shown in *J* of Fig. 3 or in Stoerk's Fig. 18 were drawn out to form a long loop of Henle, retaining the structure given them in the figures, the result would be a tubule in which the descending limb of the loop would be lined by a clear epithelium to near its end, one which would show a greater diameter than the ascending limb, which would be lined by a darker epithelium. This is what he has done and was thus led to the error he has committed. That he is not justified in assuming that the uriniferous tubules presenting the stages represented in his Figure 18 and model *L* show the structure and cellular differentiation of fully developed uriniferous tubules, may be seen, I believe, in *A* and *B* of Fig. 13 and is also shown by my other models showing more advanced stages of development. It may, therefore, again be stated that reconstructions of proper stages in the development of uriniferous tubules show that the descending limb of Henle's loop is lined by a flattened epithelium and presents a smaller diameter than the ascending limb, which is lined by a cubical or short

columnar epithelium, confirming thus the generally accepted view of the size and structure of the two arms of Henle's loop.

PECULIARITIES OF FORM PRESENTED BY THE FIRST DEVELOPED URINIFEROUS TUBULES.

Before leaving the discussion of the earlier stages in the development of the uriniferous tubules, mention may yet be made of differences in development presented by tubules which develop from the first few generations of renal vesicles, when compared with those which develop from the later generations. Such differences of development are expressed mainly in the relative degree of development shown by the different parts of the tubules which are first formed, when these are compared with those which develop later. It should, however, at the beginning be stated that such differences in development as shall here be mentioned are not of such a character as to form exceptions to the statement that the uriniferous tubules which develop from the various generations of renal vesicles present essentially the same developmental stages as have been given in the preceding pages. As has previously been stated, tubules which develop from the earlier generations of renal vesicles present in the S-stage and for some little time after, when their configurations are taken as a whole, a more rounded form than do those which develop from later generations of renal vesicles. This difference in form when the one type is compared with the other, is also observed in tubules of the respective types in which the anlagen of the different parts of the uriniferous tubule may be clearly made out in that the first developed tubules present at this stage, an irregularly spherical mass when the developing Malpighian corpuscle and the tubular complex are taken together and considered as a whole. (See *E* and *F* of Fig. 5). The tubules of similar stage of development derived from later generations of renal vesicles, likewise considered, form a more elongated mass. (See *G* of Fig. 6). This, as has been stated, is in part at least due to the fact that during the earlier stages of development of the kidney, the tubular structures are relatively far apart and are surrounded by a relatively large amount of mesenchymal tissue and may thus in growth and elongation expand in all directions, while in later stages of development the tubular structures are in much closer proximity, separated by only a relatively small amount of mesenchymal tissue; this juxtaposition to surrounding tubules influencing the direction of their growth. In their further development, tubules which are developed from the first few generations of renal vesicles are characterized by a relatively early and marked elongation of those portions of the tubule destined to form the

proximal convoluted portions, so that this portion forms a prominent part of the entire tubule for a certain period of its development, as may be seen in reconstructions and in sections. The epithelium lining this portion of the tubule as this elongates differentiates into one showing cells with clear protoplasm and nuclei in basal position as described for the proximal convoluted tubule. The loop of Henle of these tubules for a certain period of their development remains relatively short, when their length is compared with that of the proximal convoluted portion. These tubules generally show throughout, but especially in their proximal convoluted portions, a greater diameter than tubules which develop later, the lumen of the proximal convoluted portion being especially wide. Their Malpighian corpuscles are also relatively large. In tubules which develop from renal vesicles that follow the first few generations of these, the loop of Henle elongates at a relatively early period of their development so as to form a prominent portion of the tubule at a time when the proximal convoluted portion shows only a few relatively short coils; the proximal and distal convoluted portions taken together form thus a smaller mass than is formed by similar portions of the tubule first formed at a time when each type presents a loop of Henle of about the same length. This gives the two types of tubules when seen in reconstructions a characteristic form, and enables a distinction between them. To characterize these differences of form more clearly, reference is made to the models reproduced in Figs. 14 and 16. In *C* and *D* of Fig. 14, are shown two tubules reconstructed from the kidney of a rabbit embryo measuring 3.5 cm. They are from the layer of tubules situated nearest the pelvis of the kidney and are therefore of those which are first formed. Attention is called to the length and thickness of the proximal convoluted portion of each of these tubules and to the relative shortness of the loop of Henle. In *A* and *B*, are shown two tubules, presenting different stages of development, reconstructed from the same kidney and are representative of tubules the Malpighian corpuscles of which are situated nearer the periphery of the kidney and were therefore differentiated later than the Malpighian corpuscles and tubules of which *C* and *D* are types. In the second type of tubules, the loop of Henle is relatively long, when compared with the proximal convoluted portion; in *A*, of about the same length as the loop of tubules *C* and *D*, while the proximal convoluted portion of tubule *D* measures 1.5 mm., of *C*, 1 mm., and of *A* only 0.5 mm. The difference in shape presented by the two types of tubules is apparent from the figures. The Malpighian corpuscles and coiled portions of the uriniferous tubules situated nearest the pelvis of the kidney form a distinct layer, just above the mesenchyme which surrounds the

pelvis, which is readily recognized in kidneys of embryos of about the middle of foetal life; the sections of proximal convoluted portions of these tubules form the greater part of this layer in such preparations. The ap-

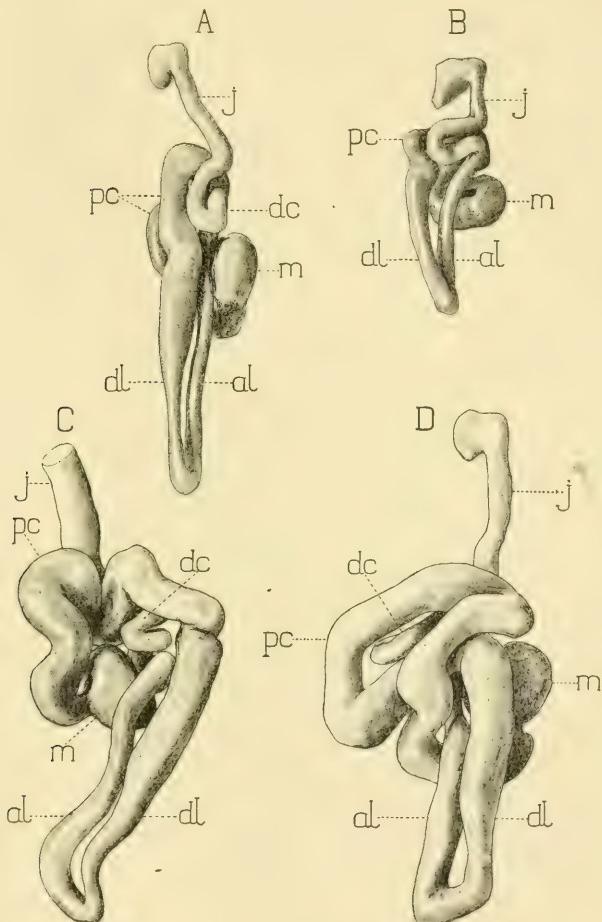


FIG. 14. Tubules from the kidney of a rabbit embryo of 3.5 cm. length (No. 8). $\times 100$. *m*, Malpighian corpuscle; *pc*, proximal convoluted portion; *dc*, distal convoluted portion; *dl*, descending limb of Henle's loop; *al*, ascending limb of Henle's loop; *j*, junctional tubule; *C* and *D* are representative of the first few generations of tubules. *A* and *B* of later generations.

pearance presented by a section of the kidney at this stage of development is shown in Fig. 15, in which is given a portion of one of the sections of the series of cross sections from which the models shown in Fig. 14 were made. The sections of the tubules and Malpighian corpuscles designated by the

letter *a*, belong to a tubule of the type shown in *C* and *D* of the previous figure; tubule *b* is the one shown in *A* of that figure. In the remainder of the section may be seen renal vesicles and tubular anlagen in various stages of development. This portion of the sections which constitutes the neogenic and subneogenic zones, stains more deeply than the portion containing the well developed proximal convoluted tubules; these, by reason of the fact that they are lined by a differentiated epithelium (as previously de-

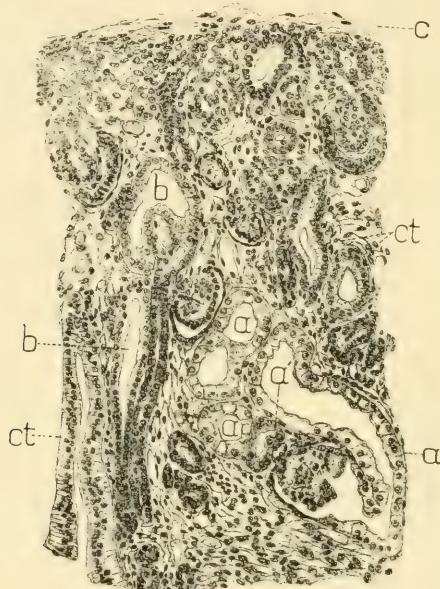


FIG. 15. A portion of a cross section through the kidney of a rabbit embryo of 3.5 cm. length (No. 8). $\times 100$. *a*, sections of proximal convoluted portion and Malpighian corpuscle of a uriniferous tubule representative of the first formed tubules of this kidney; *a'*, section of ascending limb of Henle's loop of the same; *b*, tubule shown in *A* of Fig. 14, representative of a later generation of tubules; *ct*, collecting tubule; *c*, capsule.

scribed), the protoplasm of the cells of which stains faintly in eosin and erythrosin, form a zone which stains less deeply than the more peripheral portions containing the tubular anlagen with undifferentiated epithelium of an embryonic character. In Fig. 16, are shown two uriniferous tubules, *A*, from the kidney of a cat embryo of 4 cm. length; *B* from the kidney of a cat embryo of 6 cm. length; both are representative of types of tubules found nearest the pelvis of the respective kidney and show more advanced stages of development than are shown by tubules *C* and *D* of Fig. 14. Tubule *A* measures 3.75 mm., of which 1.9 mm. falls to the proximal convoluted portion, the entire loop (ascending and

descending limbs) measuring .75 mm. The length of tubule *B* is 5 mm., of this 2.65 mm. falls to the proximal convoluted portion and 1.5 mm. to the entire loop of Henle. In these tubules, as is no doubt apparent from the figures and measurements given, the proximal convoluted por-

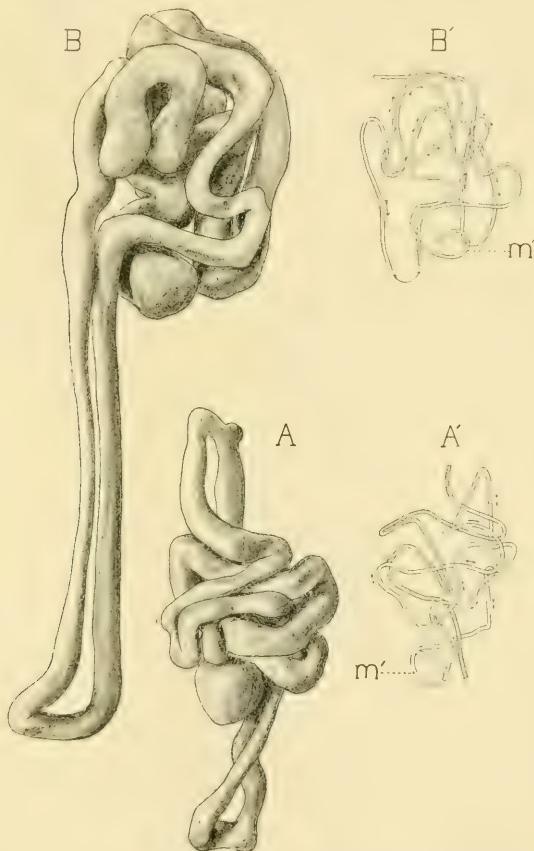


FIG. 16. Two uriniferous tubules, *A*, from kidney of cat embryo of 4 cm. length (No. 6); *B*, from kidney of cat embryo of 6 cm. length (No. 8). *A'*, *B'*, figures showing the course of the proximal and distal convoluted portions of the two tubules; the tubule representing the distal convoluted portion is shaded; *m'*, Malpighian corpuscles.

tions form the more prominent part, a little over half the length of the entire tubule. Similar observations may be made on the kidneys of human embryos of about the third and fourth month; although the differences of size and shape of the tubules which develop first when compared with those which develop later are not so marked in the human

embryo as in those of the cat and rabbit; they are, however, of similar character and of sufficient degree to merit recognition.

In Fig. 17, are represented three stages in the development of uriniferous tubules as observed in the pig embryo. My observations on the

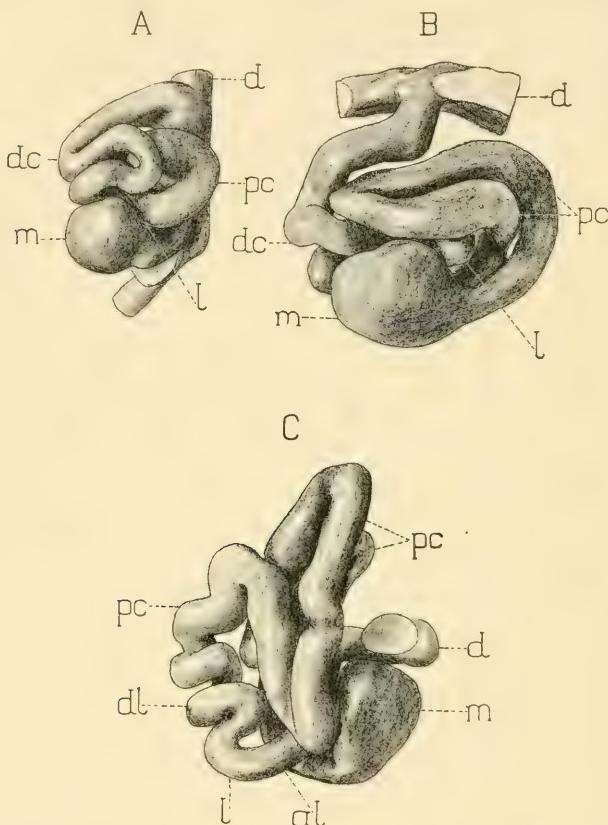


FIG. 17. Three uriniferous tubules from kidneys of pig embryos. $\times 133$. A and C, from embryo of 2.8 cm. length (No. 8). The latter is representative of the first generation of tubules, situated nearest the pelvis and is shown from the Malpighian corpuscle to the first coil of the distal convoluted portion; the former represents a much less developed tubule, one situated nearer the periphery of the kidney; B, one of the most fully developed tubules from the kidney of an embryo of 2.4 cm. length. *m*, Malpighian corpuscle; *pc*, proximal convoluted portion; *dc*, distal convoluted portion; *l*, anlage of loop of Henle; *dl*, of descending limb; *al*, of ascending limb; *d*, collecting tubule.

development of uriniferous tubules in pig embryos are not as yet complete, owing to the lack of suitable material showing the older stages of development. They have for this reason not received more than casual mention in the preceding pages. A fuller treatment is reserved for another contribution, in which the development of the collecting tubules, more

particularly the development of the so-called arcades of the collecting tubules discussed and figured by Von Ebner (p. 1105) will receive consideration. The tubules presented in Fig. 17 have been added, as they illustrate very clearly the fact that the first few generations of tubules of kidneys of pig embryos show a relatively very early development of the proximal convoluted portions and of the Malpighian corpuscle, while the loop of Henle develops at a relatively late period; tubule *B*, for instance, has attained a length of 1.5 mm. and presents a fully developed Malpighian corpuscle, at a time when the bend which is destined to form the loop of Henle (recognized by the fact that the differentiated epithelium of the proximal convoluted portion extends to nearly the top of the bend,) may just be made out. In tubule *C*, the portion which is recognized as the anlage of the loop of Henle presents a number of coils instead of being a relatively straight limb—an appearance which I have observed only in pig embryos. It may here be stated that the formation of the renal vesicles and the differentiation of the S-shaped stage of the uriniferous tubules is for pig embryos essentially the same as for cat, rabbit, and human embryos, as discussed and figured in these pages.

Hamburger's observations are of interest in this connection. In discussing the differences in form presented by the anlagen of uriniferous tubules in younger and older stages, he states that "the size of the anlage is different in younger than in older embryos and for the same stage of development it will generally be found that they are larger in the former than in the latter; especially is this true of the pseudo-glomeruli, which are especially large in young embryos." This, as may have been seen, I have confirmed, adding that they differ, not only in size, but also in shape. He further describes characteristic differences in the development of uriniferous tubules in simple kidneys when compared with those of lobulated kidneys. To quote further: "In animals with simple kidneys (mouse, rat,) the loop of Henle attains considerable development before the Bowman's capsules become closed so as to form a sphere and this is as true of the first formed anlagen as for those which develop later. In animals with lobulated kidneys ('zusammengesetzten Nieren') I have found a different condition; in them, the coiled tubules first formed attain considerable length and the Malpighian corpuscles, full development, before a loop of Henle may with certainty be discerned; the later development, however, is as in simple kidneys." His description of the development of the earlier generations of uriniferous tubules, as observed in embryos of animals with lobulated kidneys (human, pig,) coincides with that here given. In embryos of the cat and rabbit (animals with simple kidneys), I have also observed, as has been stated, a difference

between the tubules first formed and those which develop later. As I have not reconstructed the uriniferous tubules of embryos of the mouse and rat, I am not in a position to judge Hamburger's observations as concerns these; he is, however, in error when he generalizes from such observations. He further states that "In young embryos of species with lobulate kidneys (human, pig, beef), the most fully developed Malpighian corpuscles are absolutely larger than in older embryos and this is also true of the diameter of the tubuli contorti. In animals with simple kidneys (mouse, rat), this not the case." In this also, my own observations do not confirm him, as in embryos from both types of animals I have found the Malpighian corpuscles and the proximal convoluted portions (tubuli contorti) of the first formed tubules larger than of those which develop later; in the rabbit, this is especially so. (See Fig. 14.) In a discussion of his observations, Hamburger states that it may be possible that in tubules showing early development of the coiled portions and Malpighian corpuscles with absence of a distinct loop of Henle, these may develop as the kidney development proceeds. "Since without doubt, however, the first generation of coiled tubules of lobulated kidneys degenerates, the supposition is permissible that it is particularly the atypical tubules that disappear; conclusive evidence of this, I am however unable to present." Riedel (I quote from Hamburger) who also recognized the large size of the Malpighian corpuscles and tubuli contorti of the first formed tubules, states that these become smaller as development proceeds. Kölliker, whose description is based largely on rabbit embryos, quotes Riedel (page 952) as describing a degeneration of the Malpighian corpuscles and tubuli contorti first formed, for which he states there is no evidence. Emery, on the other hand, who studied the development of the kidney in goat embryos, states that he did observe evidence of a breaking down of the first formed tubules. Hamburger attempts to harmonize the conflicting views of Kölliker and Emery by stating that in embryos of animals with simple kidneys, there is no evidence of a breaking down (*zu Grundegehen*) of the first formed tubules, while in embryos of animals with lobulated kidneys, it is the atypical tubules which atrophy and disappear. My own observations lead me to say that neither in embryos of animals with simple kidneys nor in those with lobulated kidneys have I obtained evidence which would lead me to conclude that any of the uriniferous tubules atrophy and disappear in the course of development. Tubules with relatively short loop and well developed Malpighian corpuscles and proximal convoluted portion, I have observed in embryos of animals with simple and with lobulated kidneys; in such tubules, as will be stated in presenting the more advanced stages of devel-

opment of uriniferous tubules, the loop of Henle elongates at a relatively late period, developing and differentiating as do the loops of other tubules. I agree, therefore, with Minot when he states: "Some authors have maintained that there is an atrophy of some of the tubules of the foetal kidney, but I agree with Golgi in believing that of this there is no valid evidence." In offering an explanation for the difference in the development of the first generation of uriniferous tubules, when these are compared with those which develop later, which difference may be characterized as consisting of a relatively early development of the Malpighian corpuscles and the proximal convoluted portions and a relatively late development of the loop of Henle of the former when compared with the latter, attention may be called primarily to the simple mechanical condition which prevents an elongation of the loop of Henle of the first formed tubules. The Malpighian corpuscle and tubular portion which are first formed develop near the developing pelvis of the kidney, the former being separated from the latter by a relatively narrow zone of mesenchyme. As the tubules proceed in their development, their loops of Henle soon reach the epithelium of the growing pelvis and the denser mesenchyme immediately surrounding it. The loop of Henle of tubules *C* and *D* of Fig. 14 and *A* and *B* of Fig. 16 reach to the pelvis of the respective kidneys. The ends of such loops are often seen bent to one side or the other, as though attempting to avoid the obstruction which prevents their further elongation, and they are often slightly folded and twisted, the end of the loops being bent so as to project toward the periphery of the kidney, as for instance in tubule *A* of Fig. 16. Sections of developing kidneys of pig embryos of about 2.5 to 5 cm. length (the oldest stage available for me) show this in a very characteristic manner. In the series of sections from which the model shown in *C* of Fig. 17 was made, the Malpighian corpuscle was separated from the pelvis of the kidney by a distance which is about two-thirds the length of the diameter of the corpuscle; a large collecting tubule which opens into the pelvis by a funnel-shaped expansion is nearly in contact with the coiled tubular segment designated in the figure as the descending limb of the loop. There is obviously here no opportunity for the formation and the elongation of a loop with relatively straight and nearly parallel limbs. The fact that so little mesenchyme separates the tubules and the Malpighian corpuscles from the pelvis of the kidney, and the shortness of the loop of Henle at a time when the proximal convoluted portions are well developed, also the fact that the loops of Henle, so far as developed, are not present in the form of straight tubules, but as coiled tubules, so that only cross or oblique sections of them are obtained, give

to sections of kidneys of pig embryos of these stages a very characteristic appearance, resembling somewhat the appearance presented by sections of the mesonephros, and making it easy to distinguish them from sections of kidneys of cat, rabbit, and human embryos of corresponding stages of development.

The fact that the anlagen of tubules which are first formed are relatively far apart, the growth of the tubules developing from them being thus for a time not materially interfered with, may be mentioned as a possible reason for the early development and elongation of the proximal convoluted portions of these tubules, certainly an explanation of the fact that these tubular portions are coiled more in the horizontal plane than similar portions of tubules which develop later.

Malpighian corpuscles of a stage of development in which they present the form and cellular differentiation shown by these structures in post-foetal life, and proximal convoluted portions of tubules in which the epithelium has become differentiated so as to present the structural appearances presented by it in more fully developed tubules, even though such corpuscles and tubular segments are found in early stages of kidney development, may be thought to have assumed functional activity. If may be suggested, therefore, that in the uriniferous tubules which are first formed, those portions which are especially concerned with the function of excretion are rapidly differentiated to perform this function, while the other portions which are not especially concerned with the function of excretion are developed more slowly. Although there is as yet no unanimity of opinion as to the special functions to be ascribed to the different parts of a uriniferous tubule, assuming that the differences of shape and structure observed in the epithelium lining the different parts of the uriniferous tubules postulates a difference of function for the several parts lined by the especially differentiated epithelium, the generally accepted view is that the Malpighian corpuscles and the proximal convoluted portions of the uriniferous tubules are more particularly concerned with the function of excretion. The assumption, therefore, seems justified that it is for this reason, and not alone owing to mechanical conditions, that the Malpighian corpuscles and especially the convoluted portions of the first-formed uriniferous tubules are developed and differentiated relatively early, so as to enable the permanent kidney, at an early stage in its development, to assume an excretory function.

LATE STAGES IN THE DEVELOPMENT OF URINIFEROUS TUBULES.

The further growth and development of uriniferous tubules, after a stage in which all the parts show a characteristic differentiation (as for

instance seen in the tubule of Fig. 13) consist primarily in an elongation of the tubular portion, this affecting all its parts, but especially the loop of Henle. The data gained by me concerning the later stages in the development of uriniferous tubules are confined to a large extent to those obtained from observations on older embryos of cat and rabbit, the kidneys of which are of relatively simple type, with only one Malpighian pyramid. The disposition of the collecting tubules and other tubular elements of the medulla of such a kidney is such that both in series of cross and longitudinal sections a certain few will be found in which the plane of section is parallel or very nearly parallel to certain of the collecting tubules and loops of Henle. Nearly every such series will show here and there a loop, one or the other arm of which is cut for nearly its entire length, and, by using this as a starting point, I have generally succeeded in tracing out the remainder of the tubule in question. In the human kidney of the later periods of foetal life, the conditions are complicated by reason of the fact that the cortex is divided into a number of primary and secondary lobules, and even when one lobule is used for sectioning I have found it quite impossible to orient the block in such a way as to obtain long segments of either of the two arms of the loop of Henle, especially for the upper regions of the medulla. In this region the tubular elements very generally appear in oblique section and are separated in the kidneys of human embryos of the 8th and 9th months by a relatively small amount of interstitial tissue, which makes the tracing of a single tubule through this region and, as is often necessary, through a long series of sections a matter of difficulty. From a number of partial reconstructions which were made, I am led to believe that the observations made on uriniferous tubules of the older embryos of cat and rabbit (especially the former) may be accepted as presenting the conditions shown by uriniferous tubules of human embryos of the later months of foetal life. My observations on the later stages of development of uriniferous tubules, I shall present by giving a brief description of certain tubules showing these, which have been reconstructed in full and which are here figured. In Fig. 18 are shown two uriniferous tubules, the Malpighian corpuscles of which are situated in about the middle zone of the cortical portion of the kidneys from which they were reconstructed and may, therefore, be taken as representative of tubules which were differentiated after the first formed tubules of the respective kidneys. Tubule A is from the kidney of a cat embryo obtained a few days before birth. The length of this tubule is 4.1 mm., of which 1.75 mm. falls to the proximal convoluted portion, 1.65 mm. to the entire loop of Henle (the measurement here beginning and ending with the level of the lower border of the

Malpighian corpuscle) and .7 mm. to the distal convoluted portion and the junctional tubule. As may be seen from the figure, more clearly from the key, the proximal convoluted portion presents one prominent

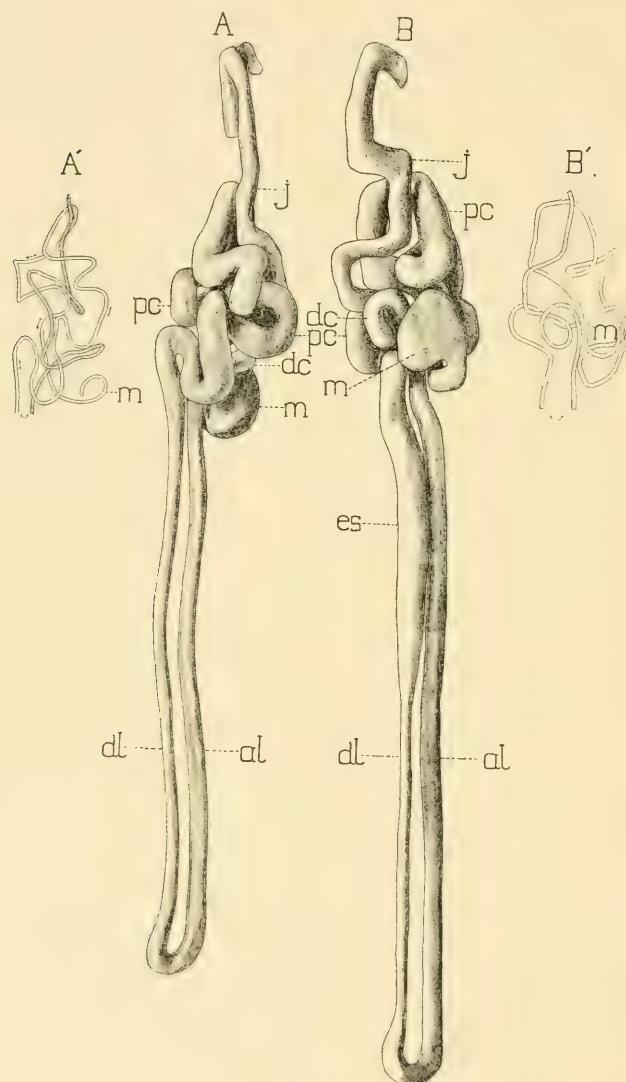


FIG. 18. *A*, uriniferous tubule from kidney of cat embryo obtained a few days before birth (No. 9); *B*, tubule from kidney of rabbit embryo 6.5 mm. in length (No. 13). $\times 100$. *A'*, *B'*, keys giving course of coiled portions of tubules, distal convoluted portions shaded. *m*, Malpighian corpuscles; *pc*, proximal convoluted portions; *es*, end segment; *dl*, descending limb; *al*, ascending limb of Henle's loop; *j*, junctional tubule.

primary loop, which extends toward the periphery, and numerous secondary loops. The distal convoluted portion is practically enclosed within the coil formed by the proximal convoluted portion. The proximal or descending arm of the loop, as the sections show, is lined nearly throughout—from a little below the level of the Malpighian corpuscle to near the loop proper—by a flattened epithelium, while the distal or ascending arm, with the loop itself, presents a slightly larger diameter and is lined by a cubical epithelium, and on reaching the coiled portion passes in front of the first part of the proximal convoluted portion, near the corpuscle. Tubule *B* of this figure is from the kidney of a rabbit embryo of 6.5 cm. length. Its length is 4.2 mm., of which 1.6 mm. falls to the proximal convoluted portion, 1.8 mm. to the entire loop of Henle, and .8 mm. to the remainder of the tubule. The disposition of the various parts of this tubule is similar to that shown by tubule *A*, except that the distal convoluted portion is more exposed. Attention should be called to the proximal arm of Henle's loop. About the upper half of this presents the same diameter (and structure) as the proximal convoluted portion. This portion represents the so-called spiral portion of Schachowa or the end piece (end segment) of Argutinski, and is in reality the distal segment of the proximal convoluted portion. It does not, so far as I have been able to observe, form a spiral; the term spiral tubule is therefore inappropriate. About the lower half of this loop presents the characteristic shape and structure of the descending arm—small diameter and lined by flattened epithelium. The transition from one to the other is clearly shown in the figure. The loop itself, as well as the ascending limb, presents a larger diameter than the thin portion of the descending limb and is lined by a cubical epithelium. It may here be stated that this tubule presents in a very characteristic way the shape, arrangement, and structure of the several parts found in tubules, the Malpighian corpuscles of which are situated in a zone of the cortex, above that occupied by corpuscles and coiled portions of the tubules first formed, which are nearer the medulla.

Tubule *A*, shown in Fig. 19, represents one of the most fully developed tubules from the kidney of a rabbit embryo 6.5 cm. long. It has a length of 5.8 mm., of which 2.2 mm. forms the proximal convoluted portion, 2.75 mm. the entire loop of Henle, and a little less than 1 mm. the remainder of the tubule. This tubule I regard as one which in earlier stages of development would have shown a relatively long proximal convoluted portion, with a short loop of Henle—a so-called atypical tubule (Hamburger)—and for the following reasons: The loop of Henle reaches to near the pelvic epithelium, being separated from it by

only a small amount of interstitial tissue; its Malpighian corpuscle is situated in the deepest portion of the cortex, just above its junction with the developing medulla; the prominent coils of the proximal convoluted portion have a more horizontal position and are, therefore, spread out more in a lateral direction than would be true for tubules which develop later, in which the prominent coils of the proximal convoluted portion are more prone to grow in a perpendicular direction, toward the cortex. As I have seen no evidence of the atrophy of the first formed tubules—so-called atypical tubules—with relatively short loops of Henle—it is assumed that this loop elongates as the medulla develops. The distal convoluted portion of this tubule lies upon—in front of, as shown in the figure—the coil complex formed by the proximal convoluted portion, the course of which may be ascertained by a study of the key *A'*. The descending limb of the loop is lined almost throughout by a flattened epithelium and presents a comparatively small diameter. The end segment is relatively short, forming only a small part of the limb. The loop itself and the distal limb have a diameter which is just about three times that of the greater part of the descending limb. The sharp bend shown by the ascending limb, just before the Malpighian corpuscle is reached, is due to the fact that a relatively large arterial branch occupies the space just beneath the corpuscle, the distal limb arching partly over this to reach the vicinity of the corpuscle. For this reason also the upper end of the proximal limb is separated by a greater distance from the Malpighian corpuscle than may be considered typical. With these exceptions, this tubule may be regarded as presenting in a very characteristic manner the shape and arrangement of the different parts of a uriniferous tubule, the Malpighian corpuscle of which is situated in the deepest portion of the cortex, thus of the first few generations of uriniferous tubules. Tubule *B* of Fig. 19 represents one of the most fully developed tubules of the kidney of a rabbit killed the first day after birth. Three exceedingly fortunate sections of the series of cross sections 5μ thick, into which one of the kidneys was cut, contained nearly the entire length of the proximal limb of its loop. The model of this tubule measures from the tip of the loop to where it ends in the collecting tubule four feet and four inches. On completing the reconstruction, the tubule proved to be one presenting a not quite typical arrangement of the coils of the proximal convoluted portion, these forming a configuration which is too open and too much in one plane. The cause of this is not readily made out at the stage of development here presented. The tubule shown in *C* of Fig. 14 presents almost the same relations of its parts, and for that

tubule it is evident that the somewhat atypical form assumed is due to the fact that its juxtaposition to other and slightly larger tubules is such that for purely mechanical reasons, on growing and elongating presumably in the direction of least resistance, its coiled portion was

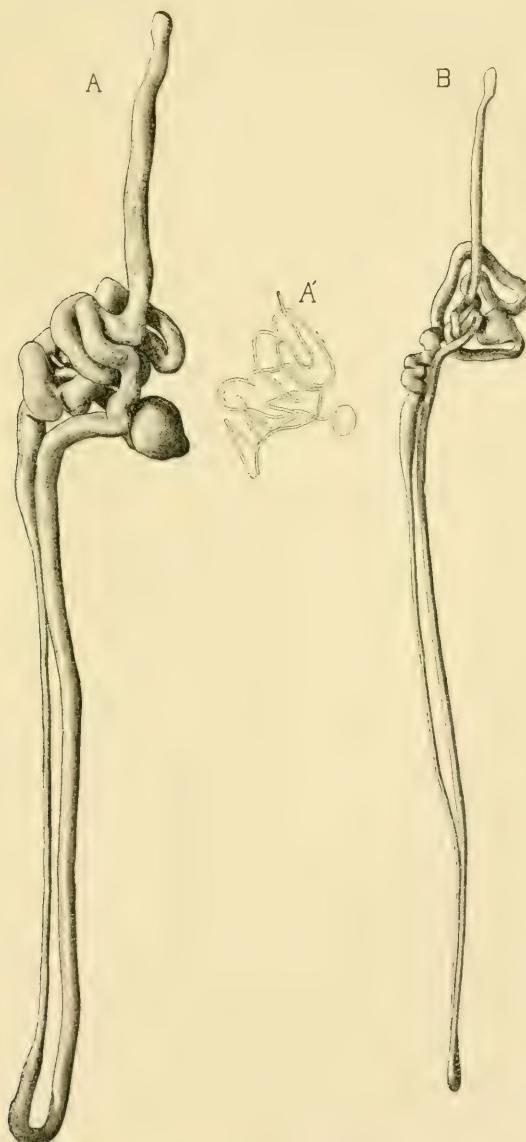


FIG. 19. Tubule A, from kidney of rabbit embryo (No. 13) measuring 6.5 cm. $\times 80$. Tubule B, from kidney of rabbit one day old (No. 14). $\times 40$.

forced to assume the form presented by it. The Malpighian corpuscle of tubule *B* is found in the second tier of corpuscles, while its loop of Henle extends nearly to the pelys of the kidney. Its entire length is 7.8 mm., of which the proximal convoluted portion forms 1.85 mm., the entire loop 4.8 mm., the distal convoluted portion 0.55 mm., and the remainder of the tubule 0.57 mm. The arrangement of the different parts of the tubule is such that its entire course may be followed in the figure; a key is therefore dispensed with. The tubule presents only a short end segment, the greater part of the descending tube to near the loop itself forming a tubule of small diameter ($12\text{ }\mu$ to $15\text{ }\mu$), lined throughout by a flattened epithelium, while the ascending limb with the exception of the lower one-sixth is lined by a cubical epithelium with faintly striated protoplasm. It is evident from the character of the epithelium which lines the loop itself and the lowest part of the two limbs (cells of embryonic character with relatively large, deeply staining nuclei) that this portion of the loop is elongating to keep pace with the enlarging and elongating Malpighian pyramid.

The tubule shown in Fig. 20 was reconstructed from the kidney of a cat embryo obtained a few days before birth; the model presents a length of four feet. The tubule itself measures almost exactly 9 mm.; 2.4 mm. belonging to the proximal convoluted portion, 4.85 mm. to the entire loop of Henle, 1.1 mm. to the distal convoluted portion, and 0.55 mm. to the junctional tubule. This tubule I regard as showing in a very typical manner the shape, arrangement, and relations of the different parts of a uriniferous tubule, especially one the Malpighian corpuscle of which is situated in the deeper portion of the cortex. The course of the proximal convoluted portion may be ascertained from the key (unshaded tubule). The curvatures of this part, as shown in the key, are, for the sake of clearness, not so pronounced as in the actual model, but are drawn with sufficient accuracy to give correctly the course of this part of the tubule. The distal convoluted portion presents two prominent loops and in its upper portion two smaller loops, in which portion there may also be observed irregularities in the diameter of the tubule. The shaded portion of the key gives the course of this portion of the tubule. The coils formed by it lie over—or in front of, as shown in the figure—the coil complex formed by the proximal convoluted portion, the prominent loop being found a little above the Malpighian corpuscle. The proximal limb of Henle's loop presents almost throughout a small diameter and is lined in this portion by the characteristic flattened epithelium. The end segment is short and presents in a typical manner the transition in shape and structure of the tubule as found

in the proximal convoluted portion to that seen in the descending limb. The loop itself and the ascending arm have a diameter which is about

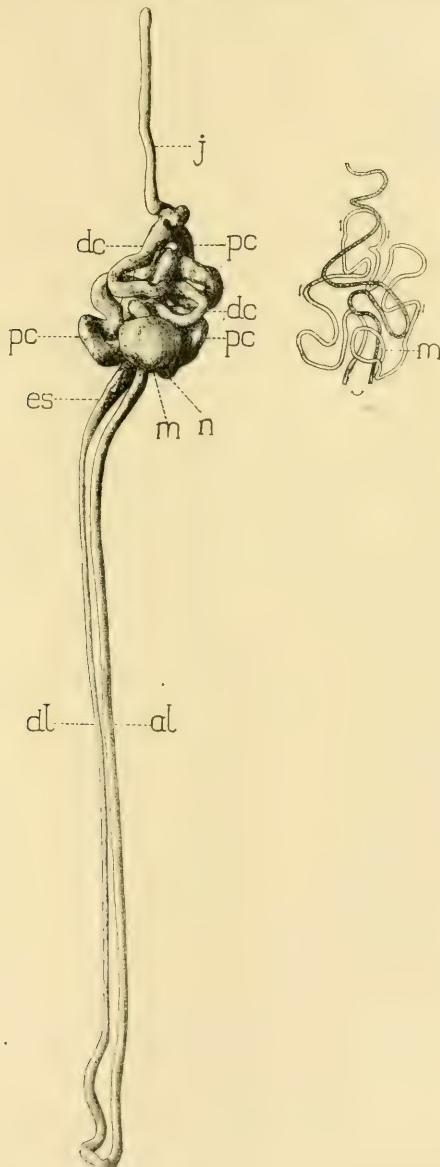


FIG. 20. Uriniferous tubule from kidney of cat embryo obtained a few days before birth (No. 9). $\times 30$. *m*, Malpighian corpuscle; *n*, neck; *pc*, proximal convoluted portion; *es*, end segment; *dl*, descending limb; *al*, ascending limb of Henle's loop; *dc*, distal convoluted portion; *j*, junctional tubule.

twice that of the greater part of the descending limb and are lined by a cubical epithelium. The ascending limb reaches the coil complex in the immediate vicinity of the Malpighian corpuscle with which it is practically in contact for about one-third of the latter's circumference. The proximal limb leaves the coil complex in close relation with the upper end of the distal limb, therefore very near to the Malpighian corpuscle. The lower end of the loop of this tubule reaches to the pelvis of the kidney, being separated from its epithelium by only a small amount of interstitial tissue.

In the tubules shown respectively in *B* of Fig. 19 and in Fig. 20, the Malpighian corpuscle is fully developed and the proximal and distal convoluted portions may be regarded as also fully developed and as having attained the size and for all practical purposes the length to which the tubular portions grow. This statement is based on the measurements made on uriniferous tubules—especially those which are developed earliest—at different stages of their development. The measurements given below show that the proximal convoluted portions at a relatively early stage in their development attain a length which is about that presented by these tubular segments in later stages of embryonic development or at birth. The following summary of certain of the measurements previously given presented in the form of a table may serve to substantiate this:

TUBULE.	OBTAINED FROM.	ENTIRE LENGTH OF TUBULE.	LENGTH OF PROXIMAL CONVOLUTED PORTION.	DISTAL CONVOLUTED PORTION.	LOOP.
Fig. 16, <i>A</i>	Cat embryo, 4 cm.	3.75 mm.	1.9 mm.75 mm.
Fig. 16, <i>B</i>	Cat embryo, 6.5 cm.	5 mm.	2.65 mm.	1.50 mm.
Fig. 20	Cat embryo just before birth	9 mm.	2.4 mm.	1.1 mm.	4.85 mm.
Fig. 14, <i>D</i>	Rabbit embryo, 3.5 cm.	2.9 mm.	1.5 mm.
Fig. 19, <i>A</i>	Rabbit embryo, 6.5 cm.	5.8 mm.	2.2 mm.	2.75 mm.
Fig. 19, <i>B</i>	Rabbit at birth	7.8 mm.	1.85 mm.	.55 mm.	4.8 mm.

The data given in the table will show, I believe, that the elongation of the uriniferous tubules, after a certain period in their development, is to a large extent due to a growth in length of the loop of Henle, the proximal convoluted portion of each tubule attaining at a relatively early period in its development approximately the length shown by this tubular segment in fully developed tubules.

To show how much of a single uriniferous tubule, representing a stage in which the Malpighian corpuscle and proximal and distal convoluted

portions may be regarded as fully developed, is contained in a selected section of a series of sections containing the whole tubule, I have inserted Figs. 21 and 22, in each of which the several parts of a single uriniferous tubule are sketched darker than the remaining portions of the sections reproduced. In Fig. 21, *A* and *B*, are reproduced portions of the cortex of two sections from the series of sections from which tubule B of Fig. 19 was reconstructed. A comparison of this figure with that showing the reconstruction will show that the plane of section was at right angles to that in which the model is reproduced. A line drawn through the middle of the Malpighian corpuscle, and the proximal convoluted portion (see *B*, Fig. 19) shows the location of the section, a portion of which is shown in *B* of Fig. 21. In this section the Malpighian corpuscle, the proximal convoluted portion soon after leaving the corpuscle and two arms of a loop of it as it comes back over the corpuscle are met with. Their relation to each other and to the surrounding structures may be seen from the figure. In *A* of the figure is shown a portion of the 13th section further on in the series. This section passes practically through the entire length of the junctional tubule (leading to the periphery of the cortex) which will indicate its position in the model; also through the coils of the distal convoluted portion, which is cut in cross section four times and through a long and a short loop of the proximal convoluted portion, escaping the Malpighian corpuscle. In Fig. 22 is shown a portion of the cortex—about the lower one-half—and the uppermost portion of the medulla of one of the sections from the series from which the model was made, which is shown in Fig. 20. The plane of the section is nearly at right angles to the model as placed on the page. The Malpighian corpuscles and tubules belonging to this uriniferous tubule are sketched more deeply than other parts of the figure. The section passes through about the center of the Malpighian corpuscle and through the upper end of the ascending limb, which may be observed as in close proximity to the corpuscle. Of the five cross sections of tubules arranged nearly in a row above the Malpighian corpuscle the first two and the last two are sections of the distal convoluted portion, the middle one and the three nearly longitudinal sections of tubular segments are of the proximal convoluted portions, the lowest one just to the left of the end of the ascending limb marks the end of the proximal convoluted portion, beyond which the tubule becomes smaller to form the descending limb. No part of the loop of Henle nor of the junctional tubule is shown in this section. I have purposely selected for figures sections of these two uriniferous tubules, since they represent tubules of very different form; the arrange-

ment of the sections of the tubules and their relation to the surrounding tubules are therefore very different. These figures may serve to show graphically the difficulties met with in attempting to predict what particular portions of any given section through the cortex of the kidney

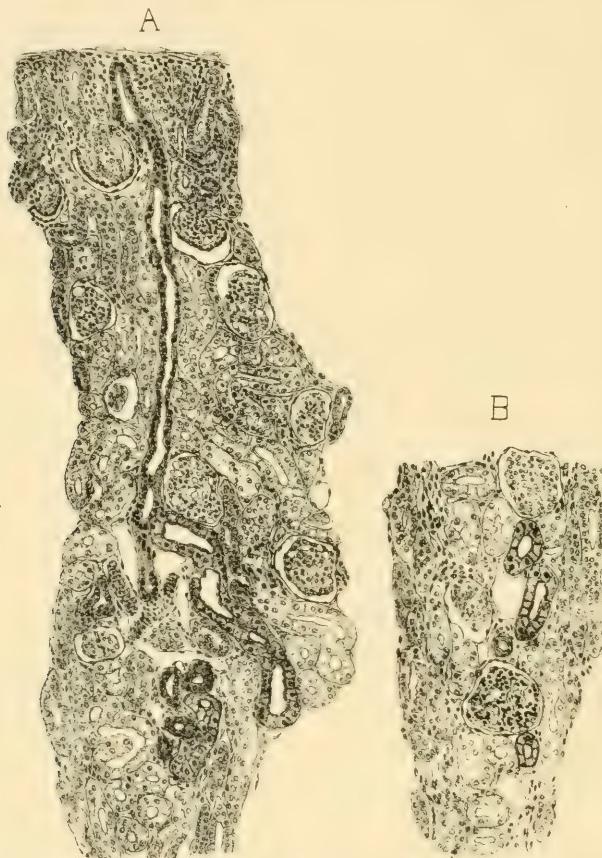


FIG. 21. A portion of the cortex of two sections of the kidney, from the series from which tubule *B*, of Fig. 19, was reconstructed. The portions of the tubule represented in these sections are sketched more deeply than the other parts. $\times 100$. For fuller description, see text.

belong to one uriniferous tubule; a question to which I shall return later.

From what has been said concerning the various stages of development of the uriniferous tubules, it may be seen that from a relatively early period in their development—from a stage in which the various parts of the tubules may be considered as differentiated to the extent

that the parts are clearly recognized—the different portions of a uriniferous tubule present certain definite relations which are not materially altered as development proceeds and are maintained in full development. In the foetal as also in the post-foetal kidney the proximal and distal convoluted portions belonging to a uriniferous tubule form a coil complex which shows certain relations to the Malpighian corpuscles of such a tubule, likewise the beginning and ending of the loop of Henle. The coil complex formed by the proximal and distal convoluted portions of a uriniferous tubule is generally situated just above its

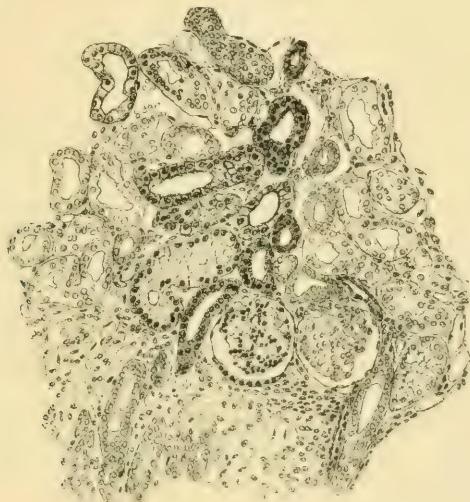


FIG. 22. A portion of the cortex and uppermost part of the medulla from one of the series of sections from which the model shown in Fig. 20 was made. $\times 100$. The parts of this tubule represented in this section are sketched darker than the other parts. For fuller description, see text.

Malpighian corpuscle, forming a more or less compact mass of convolutions, the main coils of the proximal convoluted portion lying to the inner side—toward the collecting tubule in which the uriniferous tubule terminates—the coils of the distal convoluted portion lying more to the outer side—laterally—although often partly embedded in the coils of the proximal convoluted portion or nearly completely surrounded by them. The proximal convoluted portion leaves the Malpighian corpuscle on its inner side and passes first upwards—toward the periphery of the kidney, as was also shown by Golgi—then forms coils in various directions, ultimately to return to the neighborhood of the Malpighian corpuscle and to pass toward the medulla. The main coils of the distal convoluted portion are usually found a little above the Malpighian cor-

puscle, one coil or loop generally resting upon it. The upper end of the distal arm of the loop of Henle remains from the time when it may be first recognized in close relation to the Malpighian corpuscle, either passing over it at a variable distance from the origin of the proximal convoluted portion or crossing this near the corpuscle. Hamburger, who has also observed this close relation of the ascending limb to the corpuscle, states that in a number of his preparations showing various stages of development, he found the upper end of the ascending limb of the loop of Henle in close relation with the Malpighian corpuscle and in the region where the vessels enter and leave it. This, he states, is readily explained when it is remembered that the loop of Henle is originally in the bowl of the "pseudoglomerulus," therefore in contact with the developing glomerulus; and as the loop grows in a central direction, the Malpighian corpuscle is fastened to this region by means of capillaries from the vas efferens. Golgi is quoted as saying (Stoerk) that it is the thin arm of the loop which is fastened to the corpuscle in the region of vessel exit. I agree with Stoerk in saying that neither the proximal nor distal arm shows any definite relation to the vessel porta of Bowman's capsule. That, however, especially the ascending limb of the loop returns for each tubule to the coil complex formed by proximal and distal convoluted portions into the immediate vicinity of its Malpighian corpuscle is clearly shown by all my reconstructions as well as those of Stoerk, as also the tubules obtained by maceration by Golgi and Hamburger, retaining thus in later stages of development and in post-fœtal life the relations shown in early stages of development. I have not satisfied myself that the relations become fixed through the agency of arterioles or capillaries, branches of the vas efferens, nor by an especial development of interstitial tissue in this region, although the latter factor seems now and then to play a rôle. The descending limb of Henle's loop in the region where it leaves the coil complex is generally in close relation with the upper end of the ascending limb, sometimes lying to the inner side of it, again over it, therefore also near the Malpighian corpuscle, showing in this respect also in later stages of development the relations borne in early stages of its evolution. The two limbs of Henle's loop are generally quite parallel and take quite a direct course toward the apex of the Malpighian pyramid. Certain of the loops—those belonging to the tubules which are first developed—extend to near the pelvic epithelium, the apex of the pyramid, retaining in this respect the relations shown in early stages of development, elongating as the medulla develops. Tubules of the several generations which develop later terminate at various levels in the medulla and, in

general terms it may be stated, higher up in the medulla for each successive generation of tubules, the loop of those latest formed extending only to the boundary zone of the medulla and even for the adult human kidney, remaining entirely within the medullary rays of the cortex. The descending limb of the loop is the thinner of the two and is lined by a clear, flattened epithelium; the ascending limb, showing the greater diameter, is lined by a cubic epithelium with striated protoplasm; the transition from the thin to the thicker portions of the loop occurs in the tubules reconstructed, at the lower end of the descending limb, at a variable distance from the loop itself, though generally near it. This portion of the descending limb and the loop itself show the same diameter and epithelium as the ascending limb. Schweiger-Seidel found the transition from the thin to the thicker epithelium in about an equal portion of the tubules—1, at the lower end of the descending limb; 2, in the loop itself; 3, at a variable distance from the loop in the ascending limb. His observations have received very general acceptance. Von Ebner, one of the more recent writers, who has slightly modified Schweiger-Seidel's diagram, states that "the place of thickening is inconstant; now it lies in the descending limb, now in the loop itself, often and quite regularly for loops which extend deep into the pyramid, in the ascending limb." Hamburger finds for the mouse that the loop itself and the ascending limb are lined by a granular epithelium to a few days before birth; as, however, the loop grows in length with the increase in length of the papilla, the elongation of the loop is obtained through a growth in length of the descending limb, lined by the flattened epithelium, so that the loop is formed by it, and only in the basal portion of the pyramid in the boundary zone are there found loops with the dark epithelium. In making the statement, based on observations made on my models, that the transition from the thinner to the thicker part of the loop of Henle occurs at the lower end of the descending limb, I do so with some reservation, since the models carry the development of the tubules only to the time of birth. I am aware that such statement is open to the criticism that I have applied to Stoerk's observations relating to the size and structure of the descending limb, namely, that they were made on tubules not fully developed. Yet I have observed a number of loops in series of sections of kidneys from half-grown and full-grown cats and rabbits, situated near the apex of the Malpighian pyramid, the epithelial lining of which consisted of cubic cells with granular protoplasm, while I have not found clear evidence of a loop lined with flattened epithelium. Complete reconstructions of a number of uriniferous tubules from the kidneys of adult animals are necessary to

give conclusive answer to this question. My own observations, based on reconstructions, are therefore a confirmation of Piersol's, who states, "that the relative length of the narrow part of the loop—'descending limb'—and the broader portion vary considerably, but that almost without exception the transition from the conspicuously narrow tube, lined with the peculiar spindle epithelial cells, takes place in the descending limb, often at a considerable distance before the loop itself is reached, so that both limbs in the vicinity of the loop itself are of the same diameter and lined with the same kind of epithelium."

The junctional tubule, the continuation of the distal convoluted portion, after leaving the coil complex, may pass as a relatively straight tubule or one showing a number of irregularities, for a shorter or greater distance through the cortex, ultimately arching toward the collecting tubule. The concave side of such an arch is turned toward the coil complex formed by the proximal and distal convoluted portions, this lying in fact between the junctional tubule and the collecting tubule to which a given uriniferous tubule is attached. This has also been observed by Stoerk.

In giving thus an outline of the course and the relations shown by the different parts of uriniferous tubules, it should be understood that this can be given only in a general way. For as concerns the form and relations of the proximal and distal convoluted portions and their relation to the Malpighian corpuscle and its relation to the descending and ascending limbs of Henle's loop, each tubule presents certain slight differences and variations. This the models reproduced will show. No two of the tubules reconstructed are exactly alike or even very nearly so. The several parts of the various tubules present certain characteristic relations, which, though varying more or less, are still to be recognized. The relation of the entire uriniferous tubule to the collecting tubule to which each is attached also varies, and this is quite naturally more true when considering tubules showing later stages of development. In the earlier stages of development, as may have been seen from the figures given of these, the proximal convoluted tubule leaves the Malpighian corpuscle from its inner side and passes behind the upper end of the loop of Henle and, after forming several curvatures, comes forward between the collecting tubule and the coil complex to reach again the neighborhood of the Malpighian corpuscle and to pass toward the medulla. The loop of Henle, as it leaves the coil complex, is thus in front of the Malpighian corpuscle or the first part of the proximal convoluted portion. As the proximal and distal convoluted portions elongate and form more coils, the whole tubule is often turned

on its axis to a greater or less extent, so as to bring the upper end of the loop of Henle more to the inner side of the tubule, nearer to the collecting tubule to which it is attached—in closer relation to the medullary rays. This brings the Malpighian corpuscle from a lateral to a more anterior position and the first part of the convoluted portion from a posterior to a more lateral position. This is shown by a number of models presenting the later stages of development.

Stoerk has called attention to the fact that the Malpighian corpuscles and uriniferous tubules are generally described as presenting in cross sections a round form. This, he states, is, however, not true (at least for foetal life), as his models show that they present many irregularities. This is to a certain extent true. The Malpighian corpuscles are rarely of exactly spherical form, but show here and there more or less marked depressions and elevations, or they may present a distinctly oval form. The proximal convoluted portion, especially of older stages, is often of a quite regular cylindrical form, now and then for a distance somewhat flattened, but presents as a rule throughout a quite uniform thickness. The tubule of the distal convoluted portion presents often quite marked irregularities of diameter, segments of relatively large diameter being separated by such as show a much smaller diameter; as the tubule presents now and again a sharp bend at one or the other region showing a relatively small diameter, this portion of the tubule may present a quite irregular form. Stoerk has also described and figured small cecal appendages attached to the distal convoluted portion; these I have not observed in my reconstructions.

In giving the position of the different parts of the uriniferous tubule in the kidney substance, it is generally stated that the proximal and distal convoluted portions, with the Malpighian corpuscle, are situated in the cortex between the medullary rays, forming the greater part of the cortex. This is confirmed by reconstructions. Further that the distal end of the proximal convoluted portion—the end segment of Argutinsky—enters a medullary ray and with a portion of the descending limb of the loop remains in the ray until the medulla is reached. This is true only for certain tubules, for those the Malpighian corpuscles of which are situated in the cortex above the lowermost two or three tiers of corpuscles, that is, for tubules which present a distinct end segment. The descending limb of the tubules, the corpuscles of which are situated in the lowermost two or three tiers which present indistinct end segments, passes almost directly into the medulla and cannot be said to enter a medullary ray. The loops of Henle for the greater part are found in the medulla, some, as stated, barely reaching the boundary

zone, others (the proportions I am not able to give) remaining entirely within the medullary rays. What has been said of the upper end of the descending limb of the loop of Henle applies also to the upper end of the ascending limb. The junctival tubule passes through the cortex between the rays until it arches over to reach the collecting tubule in which it terminates.

I have in connection with Figs. 21 and 22 spoken of the relations, as seen in sections, shown by the coil complex and the Malpighian corpuscles of any given tubule. A study of the sections from which the models were made has not enabled me to formulate any general law concerning this relation. This depends, as may readily be seen, even in what may be regarded as very favorable sections, entirely on the direction of the sections. Frequently the greater part of the coil complex formed by the proximal and distal convoluted portions of a given tubule may be cut in a series of sections without cutting the Malpighian corpuscle of said tubule. The relations of coils of juxtaposed tubules is such that one or more of the loops, especially of the proximal convoluted portion of one tubule, may penetrate for a longer or shorter distance into the coil complex of another tubule, so that in sections the former might readily be interpreted as belonging to the latter. Stoerk gives in Fig. 13 of his article a portion of a section of a kidney showing nephritis haemorrhagica, certain tubules of which contained red blood corpuscles; this enabled him to select the sections of Malpighian corpuscle and tubules belonging to one uriniferous tubule. The section reproduced shows a characteristic relation of corpuscles and tubules cut, such as is, however, not frequently met with, if I may judge from the sections from which my reconstructions were made. The relations there shown are more nearly approached in Fig. 22 and also in the sections from which model A of Fig. 19 was made than in other series of sections used in modelling the other older developmental stages presented.

It is somewhat hazardous to attempt an estimate of the length of a fully developed uriniferous tubule, without the possession of models showing them in full development. Since, however, the proximal and distal convoluted portions of certain uriniferous tubules attain approximately their full development before the birth of the embryo (cat and rabbit), and since the length of the loop of Henle of these tubules depends on the width of the medulla, as they extend through or nearly through the medulla, an estimate of the length of a fully developed tubule seems justifiable. The length of tubules must necessarily vary, since the loop of Henle, which for the majority of tubules forms their greatest part, must vary in length, since they terminate at different

levels in the medulla. An approximation of the length of uriniferous tubules may, therefore, be most readily made for those tubules the Malpighian corpuscles of which are situated in the deepest portion of the cortex, the loops of Henle terminate presumably near the apex of the Malpighian pyramid. The most fully developed tubule, showing a typical arrangement of parts, that I have reconstructed, is the one shown in Fig. 20. This is from the kidney of a cat embryo, obtained a few days before birth, and measures 9 mm. In one of the sections of this kidney containing nearly the entire length of the distal limb of the loop of Henle of this tubule, the width of the medulla is approximately 2.22 mm. (the junction of the cortex and medulla does not form a straight line), very nearly one-half the entire length of the loop of Henle (4.85 mm.). In sections passing through the middle of the Malpighian pyramid of the kidney of a full-grown cat, the medulla has a width of approximately 12 mm. Assuming, therefore, that the loops of Henle of certain of the uriniferous tubules of this kidney, the Malpighian corpuscles of which are situated in the deepest portion of the cortex, traverse the entire width of the medulla, and one is justified in doing this, since loops are seen in section near the very apex of the pyramid; such a loop would have a length of approximately 24 mm.; add to this the length of the proximal convoluted portion, which may be given at 2.5 to 3 mm., the distal convoluted portion about 1 mm., and the junctional tubule about 2 mm. (the width of the cortex in the sections from which the measurements for the medulla were given varies from 2.5 mm. to 3 mm.), the entire length of such a uriniferous tubule would be approximately 3 cm. Other tubules, the loops of Henle of which do not pass so deep down in the medulla, would show a correspondingly shorter length, the shortest ones measuring, if I may be allowed to estimate, 10 mm. to 12 mm.

V. Ebner quotes Schweiger-Seidel as stating that the proximal convoluted portion of a uriniferous tubule forms about one-fourth to one-fifth of the entire uriniferous tubule, while the distal convoluted portion, which is shorter than the proximal portion, is about one-seventh of the latter's length. As concerns the relative length of the proximal convoluted portion, this may be regarded as correct for tubules with relatively short loops of Henle. In tubules with relatively long loops of Henle it forms a relatively shorter portion—from one-eighth to one-tenth of the entire tubule. So far as I am able to judge from my own models and from other observations, the distal convoluted portion presents a length which is about one-third to one-fourth that of the proximal convoluted portion. If other estimates of the length of uriniferous tubules or of parts thereof are to be found in the literature, they have escaped my notice.

It is not my purpose to enter here on a discussion of the epithelium lining the different parts of a uriniferous tubule. For this the reader is referred to Disse's quite recent account, he himself having made noteworthy contributions to this point. The observations made on the sections from which the models were made lead me to recognize three distinct varieties of epithelium in a uriniferous tubule:

1. The epithelium lining the entire proximal convoluted portion, to the region in the upper part of the descending limb, where the tubule is quite rapidly reduced in size, where it assumes the shape and structure of the narrow portion of the limb. The epithelium of the proximal convoluted portion is sufficiently known to obviate an especial description of it.

2. The clear, flattened epithelium of the descending limb of the loop in the region in which it presents a small diameter. I have gained the impression that too much emphasis is put on the statement that the cells of this epithelium are thicker in the region of the nucleus, thus bulging into the lumen of the tubule, and as the nuclei do not lie opposite, but alternate in the course of the tubule, its lumen is not of the cylindrical form, but irregular—wavy as seen in sections. In sections from material which I have regarded as showing good fixation, the lumen of this portion of the uriniferous tubule presents a much more regular outline than in sections from material not so well fixed, the cells presenting throughout a more uniform thickness. The suggestion is therefore made that the description, as generally given, refers to preparations which do not present the correct structure of this portion of the uriniferous tubules. This point is reserved for further investigation.

3. The last portion of the descending limb, the loop itself, the ascending limb, the distal convoluted portion, and the junctional tubule to near the collecting tube present an epithelium which shows great similarity of form as well as structure. The lining cells show a form which is irregularly cubical or short columnar. Disse describes the epithelium of the ascending limb as showing indistinct cell boundaries, while these are more distinct in the epithelium of the distal convoluted portion; the protoplasm of the latter, although granular, is also described as being clearer. In both regions the cells show an outer dark zone which is finely striated and an inner zone which is lighter, the nuclei being placed at the junction of the two zones. In my own preparations the epithelium lining the ascending limb and the distal convoluted portion do not respectively present a structure which differs to a degree making it necessary to group them separately, and I have, therefore, placed them under one head. The uriniferous tubule, for a short though variable

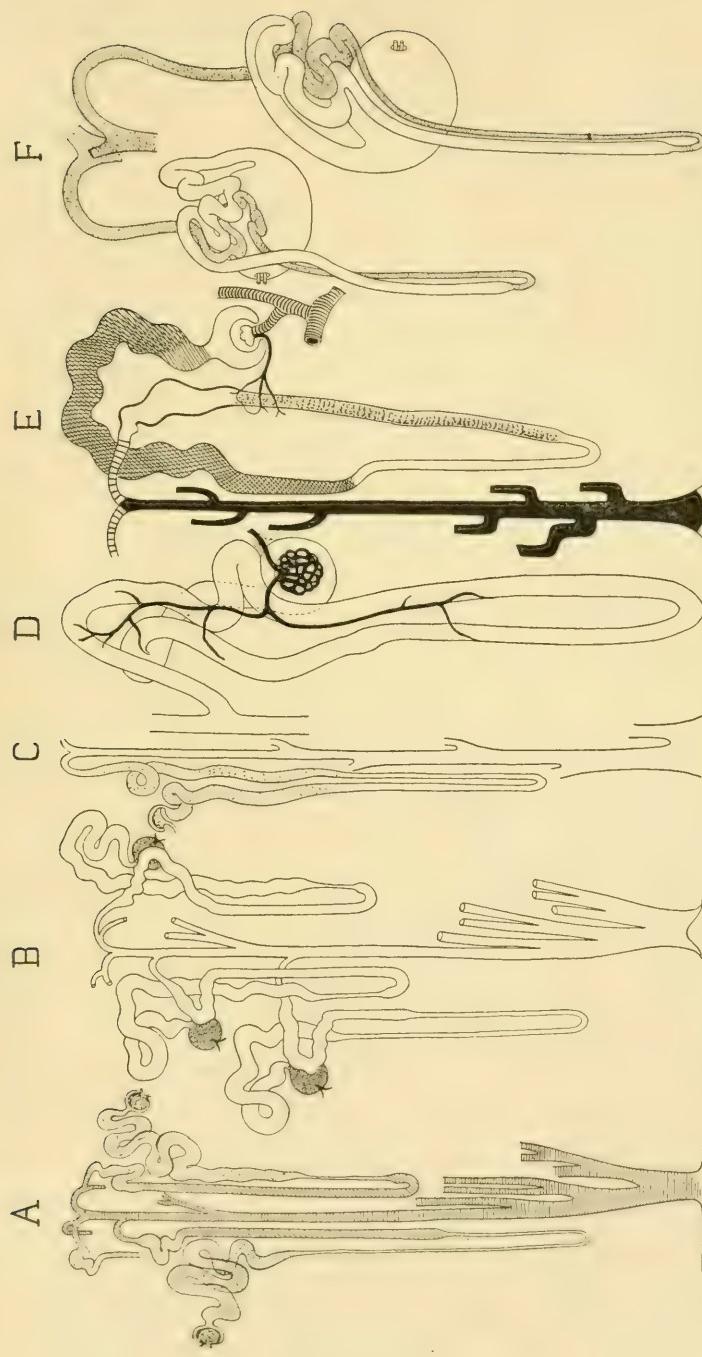


FIG. 23 DIAGRAMS SHOWING THE DIFFERENT CONCEPTIONS HELD OF THE FORM OF THE URINIFEROUS TUBULE.

A. Schweiger Seidel's diagram (as given by Stoerk, 1904, Fig. 1a); B. Von Ebner's diagram (Kölleker's Handbuch d. Gewebelehre des Menschen, Erste Hälfte, III, Bd., Fig. 1062); C. Haycraft's diagram of a uriniferous tubule of a rabbit, Fig. 13; D. Golgi's figure (Stoerk, 1904, Fig. 21); E. Disse's diagram (Bardolech's Handbuch d. Anat. des Menschen, Harn und Geschlechtsorgane, Erster Theil, VII Bd., Fig. 16); F. Stoerk's diagram (1904, Fig. 27).

distance, before reaching the collecting tubule, presents an epithelium which is like that of the small collecting tubules. This short segment may be spoken of as belonging to the collecting tubule or with equal propriety as forming part of the uriniferous tubule proper, as it is difficult to state with any degree of certainty whether it develops as an outgrowth of the collecting tubule or is differentiated with the other parts of the uriniferous tubule from the renal vesicle.

Before giving by way of a diagram or scheme the shape of a uriniferous tubule and its relation to a collecting tubule, as this presents itself to me, brief mention may yet be made of a number of diagrams of uriniferous tubules found in the literature, each of which presents certain characteristics which make it different from the others selected. These I have grouped under Fig. 23. The different conceptions of the form of a uriniferous tubule held by the authorities presenting the diagrams here given and their departure from the conception of the form of a uriniferous tubule as presented in Fig. 24 may be most readily expressed in this graphical manner.

In *A*, Schweiger-Seidel's diagram, is given inaccurately the relative position of the Malpighian corpuscle and proximal and distal convoluted portions, more especially the distal convoluted portion which is separated too much from the proximal convoluted portion. In *B*, Von Ebner's diagram, an attempt is made to bring the distal convoluted portion in relation with the Malpighian corpuscle (accepting Hamburger's observations) and to show that the first part of the proximal convoluted portion extends toward the cortex (Golgi). The diagram presents inaccuracies in the relations given to the Malpighian corpuscle and the proximal and distal convoluted portions. In *C*, Haycraft's diagram, of a uriniferous tubule of a rabbit, he presents, as is obvious, deductions drawn from the study of a tubule showing an early stage of development, shown in his Fig. 10. This, as I have stated, very probably presents parts of two tubules, there sketched as one. His diagram is wrong in the position given to the Malpighian corpuscle and as to the length, shape, and relative position of the proximal and distal convoluted portions. *D*, one of Golgi's figures, shows a uriniferous tubule at a relatively early stage of development. This is not a diagram, but the figure is introduced, since it shows quite correctly the relations of the different parts of a uriniferous tubule. *E*, Disse's diagram, one of the most recent at hand, gives incorrectly the relations shown by the ascending and descending limbs of Henle's loop to the Malpighian corpuscle and the course of the first part of the proximal convoluted portion. *F*, Stoerk's diagram, based on reconstructions of uriniferous tubules, presents a number of inaccuracies, as is

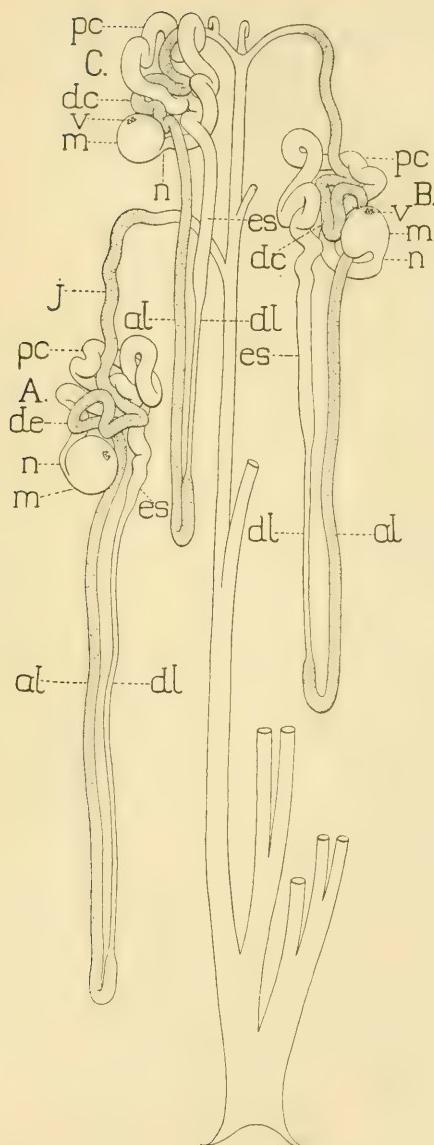


FIG. 24. Diagram of three uriniferous tubules and their relation to a collecting tubule; *A*, of a tubule, the Malpighian corpuscle of which is situated in the lowermost portion of the cortex; *B*, about the middle of the cortex; *C*, in the outer portion of the cortex. *m*, Malpighian corpuscle; *v*, vessel porta; *n*, neck; *pc*, proximal convoluted portion; *es*, end segment; *dl*, descending limb; *al*, ascending limb of the loop of Henle; *dc*, distal convoluted portion; *j*, junctival tubule; *c*, collecting tubule (Huber).

shown by my own observations on models of uriniferous tubules showing much older stages of development than the oldest tubule reconstructed by him. In this diagram, the Malpighian corpuscle is relatively too large, the course given to the first part of the proximal convoluted portion is too regular; the proximal convoluted portion is relatively too short when compared with the length given to the distal convoluted portion, but especially wrong is the diagram in the presentation given of the descending limb of Henle's loop. This, as given by Stoerk, shows essentially the same diameter as the proximal convoluted portion (in his description he gives it a similar epithelial lining), disregarding entirely that portion of the descending limb of the loop which shows a flattened epithelium.

In the diagram of Fig. 24 the loop itself of the loop of Henle is given as formed by a tubule the same as that of the ascending limb, the transition from the thinner to the thicker portion of the limb as occurring in the lowermost part of the descending limb. This is in conformity with my models. In discussing this point, I have stated that this observation was given with some reservation. I have, therefore, not called especial attention to certain of the loops shown in the diagram *A*, *B*, and *E* of Fig. 23, which are formed by the thinner portions of this structure.

In conclusion, I desire to thank Julius H. Powers and Edward G. Huber for assistance given me in the reconstructions, and Elton P. Billings and Frederic G. Johnson for assistance rendered in making the illustrations,—students of medicine in the Department of Medicine of the University of Michigan.

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Sept. 25, 1915



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